



US 20080008993A1

(19) **United States**(12) **Patent Application Publication****Kahl et al.**(10) **Pub. No.: US 2008/0008993 A1**(43) **Pub. Date: Jan. 10, 2008**

(54) **USE OF A TYPE III RESTRICTION ENZYME TO ISOLATE IDENTIFICATION TAGS COMPRISING MORE THAN 25 NUCLEOTIDES**

(86) PCT No.: PCT/JP03/05840

§ 371(c)(1),

(2), (4) Date: Oct. 16, 2006

Publication Classification(51) **Int. Cl.***C12Q* 1/68 (2006.01)*C07H* 21/04 (2006.01)*C12N* 9/00 (2006.01)(52) **U.S. Cl.** 435/6; 435/183; 435/262; 536/23.1; 536/24.2; 536/24.33

(75) Inventors: **Guenter Kahl**, Seligenstadt (DE); **Peter Winter**, Kelkheim/Ts (DE); **Detlev Krueger**, Stahnsdorf (DE); **Stefanie Reich**, Berlin (DE); **Hideo Matsumura**, Iwate (JP); **Ryohei Terauchi**, Iwate (JP)

Correspondence Address:

BIRCH STEWART KOLASCH & BIRCH
PO BOX 747
FALLS CHURCH, VA 22040-0747 (US)

(73) Assignee: **IWATE PREFECTURAL GOVERNMENT**, MORIOKA-SHI IWATE (JP)

(21) Appl. No.: 10/556,030

(22) PCT Filed: May 9, 2003

ABSTRACT

The type III restriction enzyme EcoP15I is used to isolate from cDNA of an expressed gene a tag comprising more than 25 nucleotides and capable of identifying the expressed gene, wherein the 3' end of the tag is defined by a cleavage site of the type III restriction enzyme and the 5' end of the tag is defined by the cleavage site of another restriction enzyme that is closest to the 3' end of the cDNA of the expressed gene. The tag of the invention allows accurate quantitative gene expression analysis and rapid gene expression profiling in any organism for which no expressed sequence tag (EST) database is available.

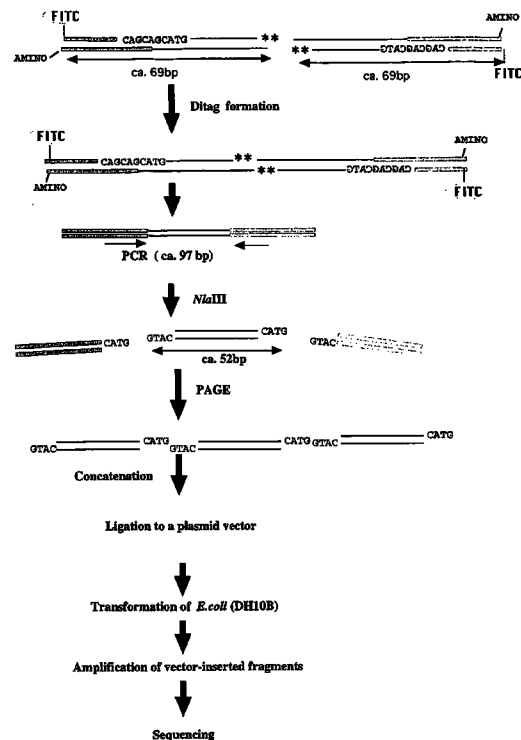
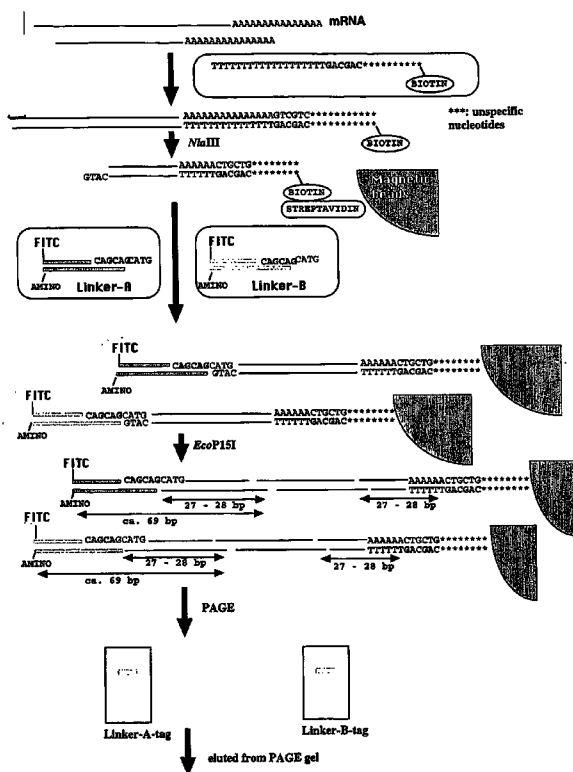


Fig. 1

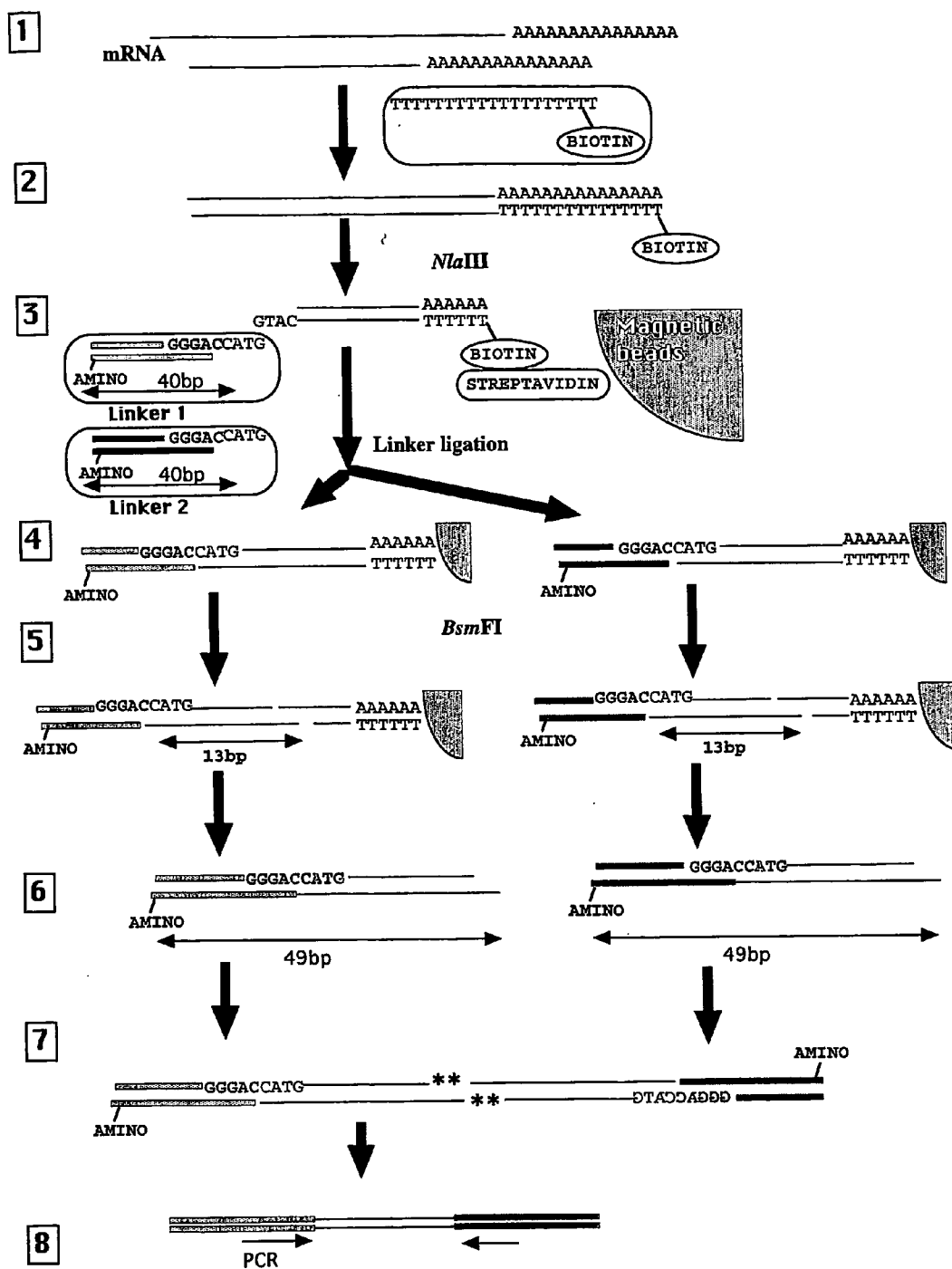


Fig.2

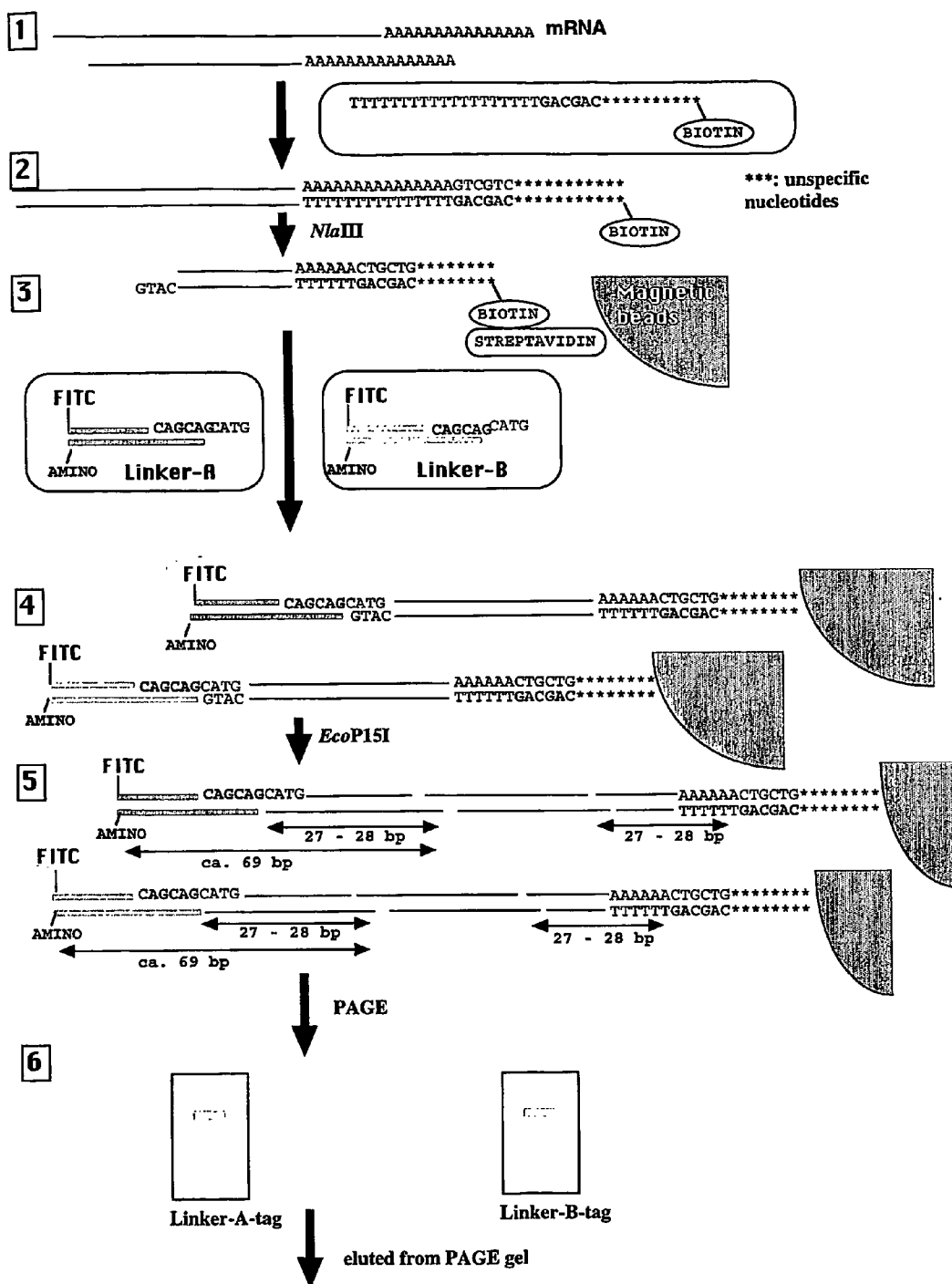


Fig.2

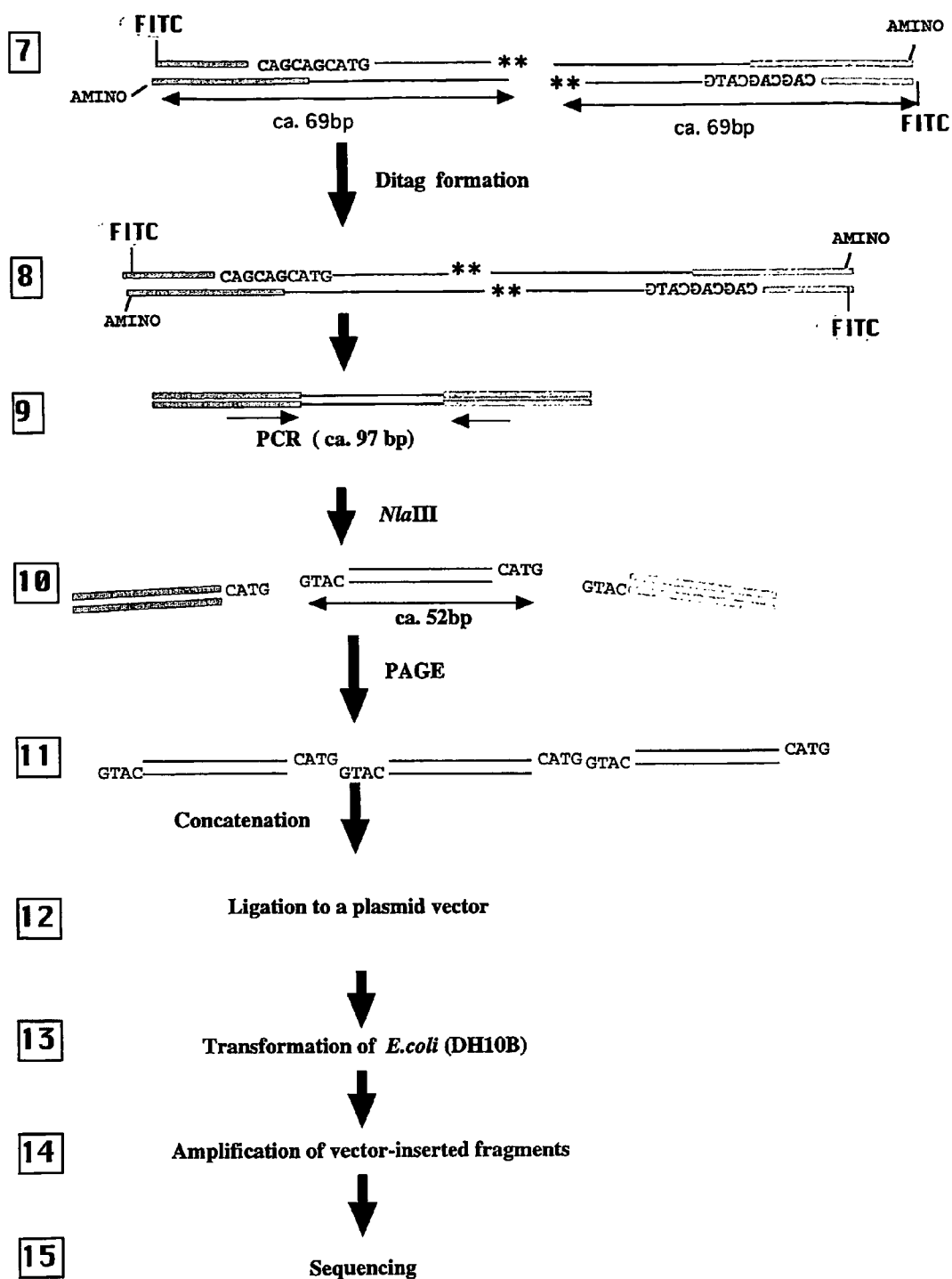


Fig.3

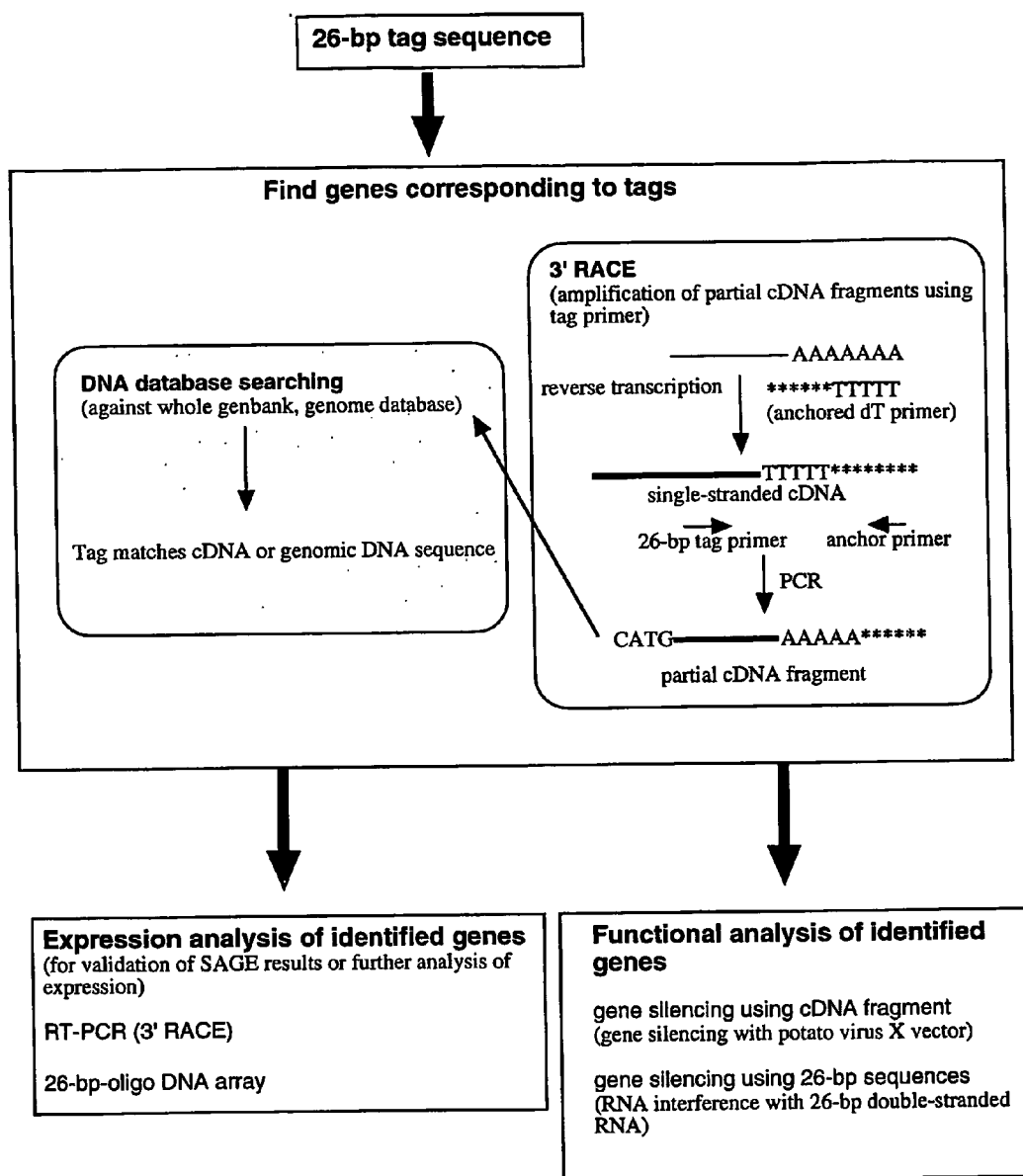


Fig. 4

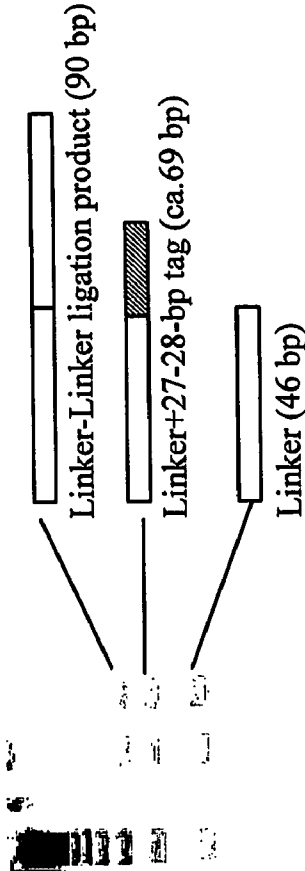


Fig. 5

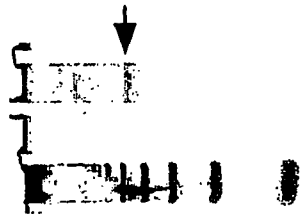


Fig. 6

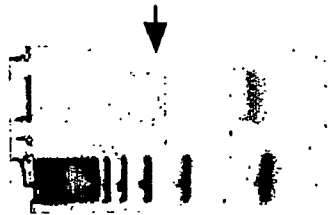


Fig. 7

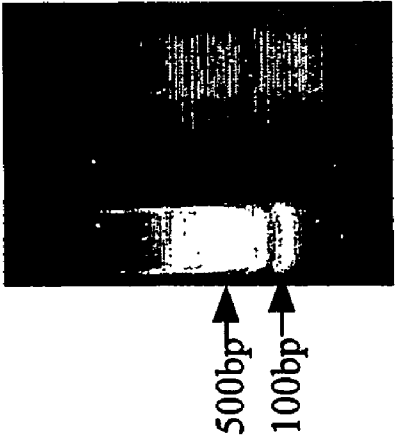


Fig. 8

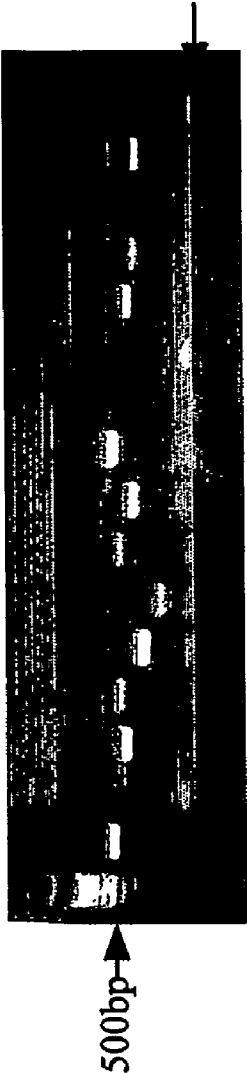


Fig. 9

Sph I site

CAGGCATGTGTAACCTCACTGGATGGAGTGGAGAAATCACCCAGGACAAATTACCATGCTGGTATTCTGCAACGTTACATT
 ACCAGTCAGTAGCTTTTCCCATGGGACAAATGTCACCTGGTTCACAGAAATGATCATCCAGCAATAATTATCATGTTTCATC
 AGCATATTTTCTAATAATTCTTTACCATCAAGCAATGATCCATCGGGGTGCTCTACATCTCTTCTTACGACGACGCAGGG
 GGCGCGGACCATGTATCAATGTATTTTATCTGTAGTCTTCAGGTTACAGATTATTACATGTAGAGTTAGAGATATTTGA
 GCTCTTAGCGTATTTTACTCAAAATCGCATGGGCGCCCTCCCGTGTGTCGTGGATCAGACATTTGTGATATATCATGGTC
 CCAGCTTACTGCAAGATCAAAACAACCTAGGCTTAATACAGCAGCATGGTCGCCCTCTCTGCTCGTGCCCGCCCGGATCGGCCA
 TTAGCATTTCTCGCATGGCGCGCGGATGGTCCCTCCCGGTACATTCATCCTGTACAGTATCTCATGCAAG

Sph I site

Fig.10

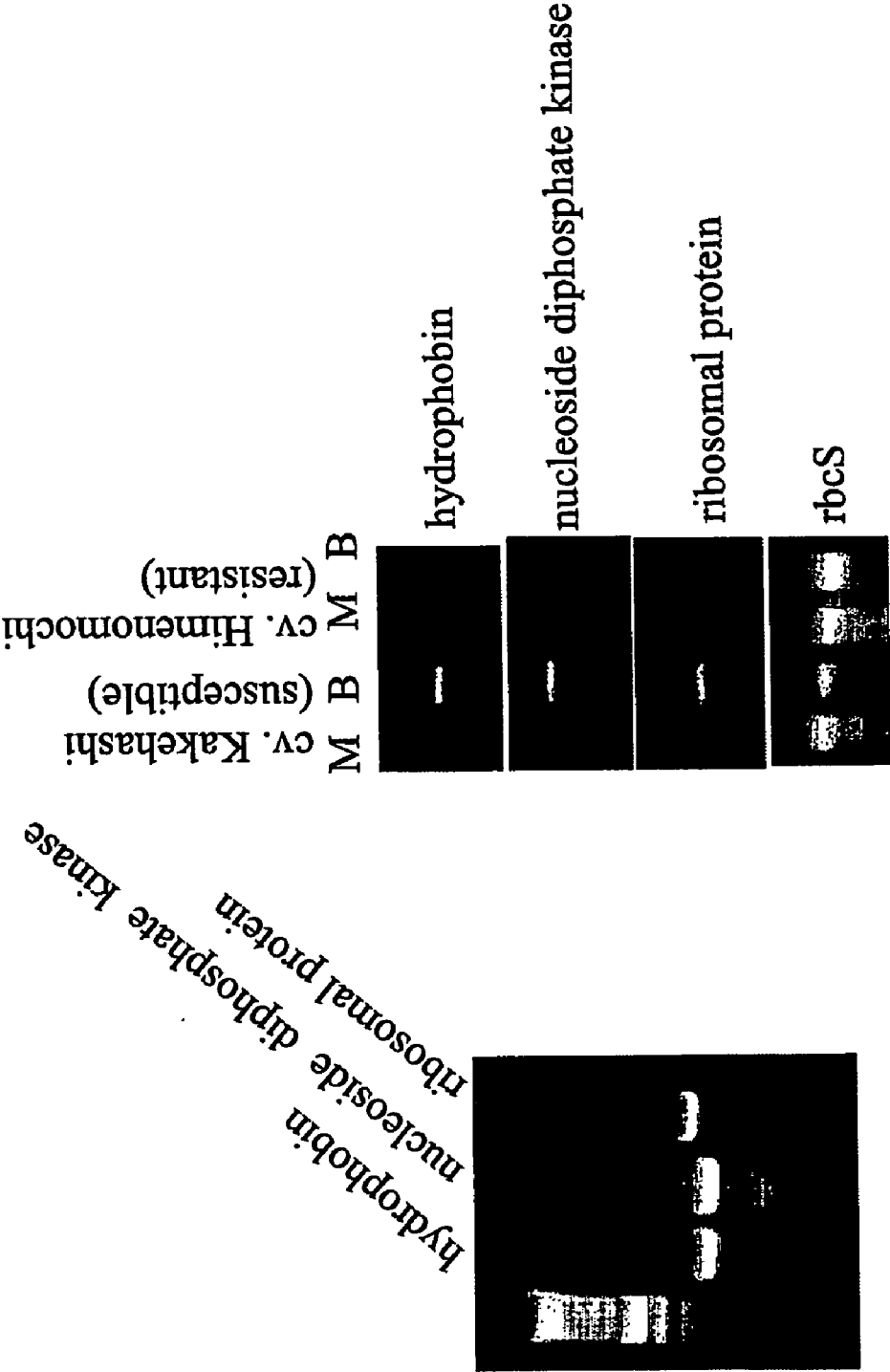


Fig.11

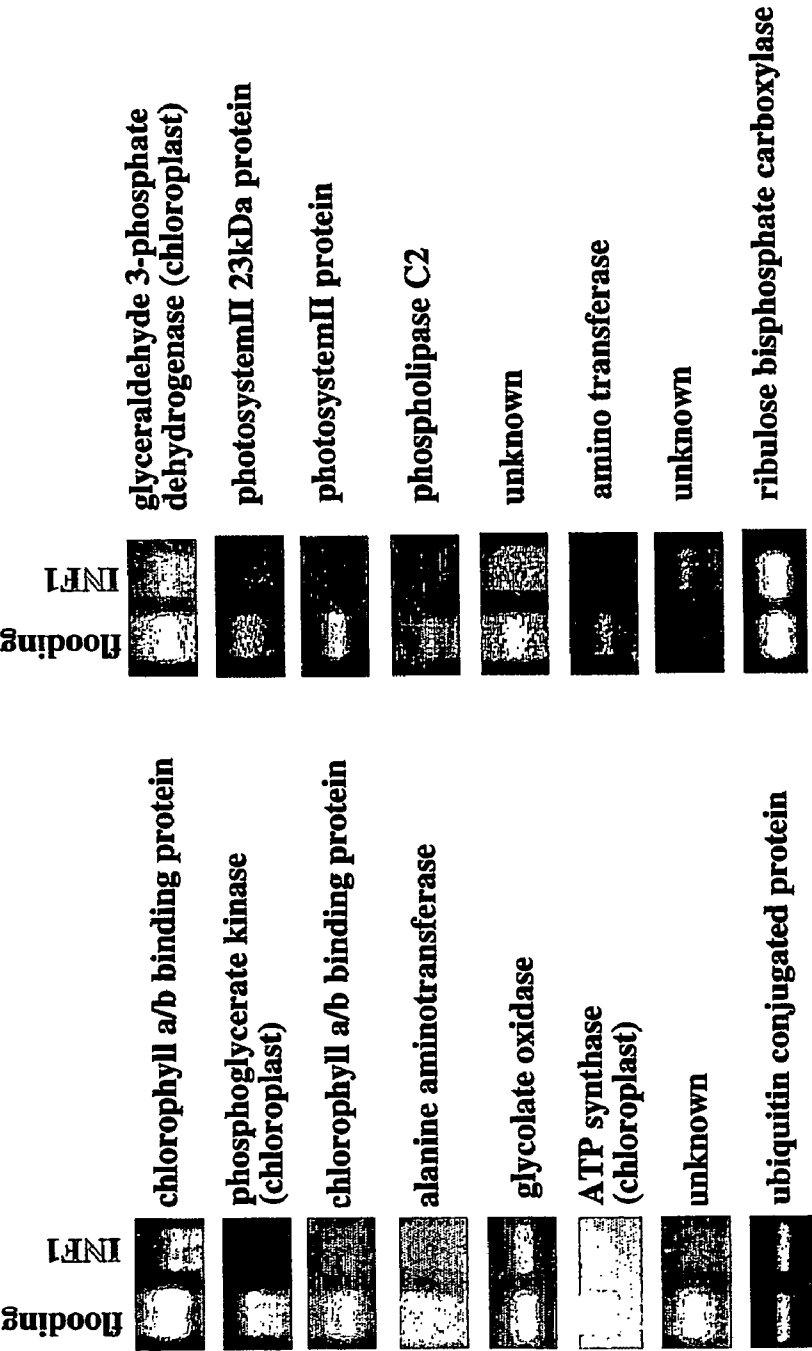
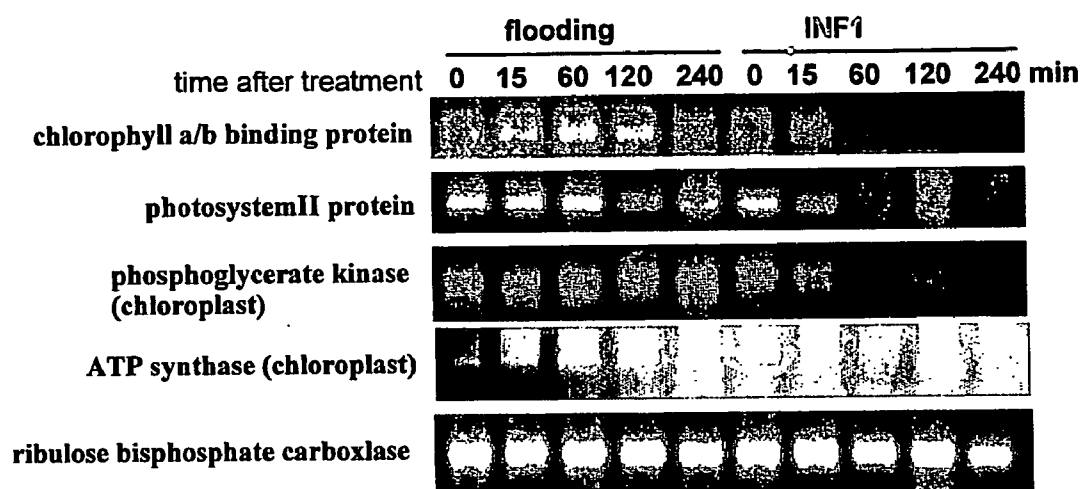


Fig. 1 2



USE OF A TYPE III RESTRICTION ENZYME TO ISOLATE IDENTIFICATION TAGS COMPRISING MORE THAN 25 NUCLEOTIDES

TECHNICAL FIELD

[0001] The present invention relates to the field of molecular biology and analysis of gene expression. In this context, it concerns the use of a type III restriction enzyme for isolating a defined region of a transcript.

BACKGROUND ART

[0002] Modification of the structure and expression of genes by genetic engineering provides enormous potential for various medical, pharmaceutical and agricultural applications. In order to detect and select target genes for engineering, screening of cells, tissues, organs or organisms in different developmental stages and under a variety of environmental conditions (such as stress) by expression profiling is extensively applied. One of the most powerful techniques for gene expression analysis is SAGE™ (Serial Analysis of Gene Expression) as developed by Velculescu et al., Science 270: 484-487, 1995.

[0003] In the original SAGE™ protocol (FIG. 1), a pool of messenger RNA (mRNA) from defined cells, tissues or organs is used as the source material, from which complementary DNA (cDNA) is reversely transcribed by reverse transcriptase using a biotinylated oligo-dT primer. The generated single-stranded cDNA is converted into double-stranded DNA, and digested with restriction enzyme NlaIII, which recognizes the sequence motif 5'-CATG-3'. Streptavidin-coated magnetic beads are used to recover the 3'-end fragments of the double-stranded cDNA. The cDNA is divided into two portions. Two linkers (Linker 1 and Linker 2) are then ligated to each of the cDNA portions. The linkers contain the sequence motif 5'-GGGAC-3'. This is the recognition site of the type II restriction enzyme BsmFI, which cleaves 13 bp apart from the recognition site in the 3'-direction. Thus, treatment of the linkered cDNAs with BsmFI releases a 13-bp fragment of the cDNA, called the "tag" sequence, together with the linker fragment ("linker-tag" fragment). Then two of the linker-tag fragments generated from two portions of cDNAs are ligated to each other in such a manner that the two tags are in adjacent position to form a ditag, followed by PCR with primers specific to the linker sequence. After removing the linker fragment by NlaIII digestion, the ditags are concatenated, and cloned into an appropriate plasmid. Sequencing of the plasmid insert shows a series of 9 bp tags flanked by the 4-bp 5'-CATG-3' sequence. Using the 13-bp tag sequence, in many cases it is possible to identify the gene from which a tag sequence originated, by consulting available expressed sequence tag (EST) databases. Thus, after sequencing thousands of tags, it is possible to count the number of each tag in the sample, and further identify the genes corresponding thereto.

[0004] The SAGE™ protocol described above is therefore an effective method to study global gene expression. However, the limited size of the tag sequence (only 13 bp) is not sufficient to unequivocally identify the gene from which the tag was derived. A single tag sequence may correspond to several different EST sequences, and may confound further analysis.

[0005] To improve the situation, a so-called "Long-SAGE™" protocol was recently developed, involving the

type II restriction endonuclease MmeI. With the use of MmeI (instead of BsmFI as in the original SAGE™ protocol), it is possible to recover 19 to 21-bp-long tags (Saha et al. Nature Biotechnology 20: 508-512, 2002).

[0006] MmeI digestion produces 3'-protruding ends (2-base protrusions). To make blunt ends after MmeI digestion before ditag ligation, one must remove the 3'-protrusion. Currently there is no single enzyme available for filling in the 3'-protrusion. Removal of the 3'-protrusion necessary for blunting results in the reduction of the length of tag sequence to 17-19 bp, causing reduction in the information contained in the tag sequence.

[0007] Therefore, in the published LongSAGE™ protocol (Saha et al., Nature Biotechnology 20: 508-512, 2002), the ends after MmeI digestion are not polished, and ligation is made between the fragments with 3'-protrusions. This means that a linker-tag fragment ligates itself only with another linker-tag fragment harboring the compatible 3'-end. This entails that ditags formed after ligation are not the results of random association of tags. This procedure theoretically skews the representation of each tag in resulting ditags, and the final result of LongSAGE™ may not faithfully reflect the abundance of expression of each gene. Therefore, LongSAGE™ is not applicable for accurate quantitative analysis of gene expression. This represents a serious drawback of LongSAGE™. For this reason, the current LongSAGE™ is only used to help annotate the 13-bp tag sequences obtained by the conventional SAGE™ protocol.

[0008] The LongSAGE™ protocol is a step forward, because it increases the possibility of sequence identification of the genes corresponding to the tags, given that EST and/or genomic DNA sequence information is available. However, for an application of the SAGE™ protocol to organisms, for which no EST or genomic DNA database is available, it is imperative to use the tag sequence as a primer to recover the longer cDNA by PCR, or as an oligonucleotide probe to screen a relevant cDNA library by hybridization-based techniques. For these purposes, tag lengths of 19-21 bp are still too short to unequivocally identify the gene from which a tag sequence originated.

[0009] Therefore, there is an acute need for a method to isolate noticeably longer tags of at least 25 bp in length, with which accurate quantitative analysis of expression can be performed. However, such a method has not been available to date.

SUMMARY OF THE INVENTION

[0010] The present invention provides a method for isolating "tag" sequences of more than 25 bp long, preferably 26 to 50 bp long, and most preferably 26 to 28 bp long, from defined positions of DNAs, thereby increasing the efficiency to reliably identify the corresponding genes by conventional SAGE™ analysis. Moreover, the gene expression profiles obtained by this method are theoretically more accurate than those obtained from LongSAGE analysis, since the ditags are made by the random association of tags. We hereinafter term this improved SAGE™ procedure with new tag fragments of more than 25 bp as "SuperSAGE™".

[0011] The method described herein is based on the use of a type III restriction enzyme, for the isolation of tag sequences of more than 25 bp in length from a defined

position of a cDNA. In the present invention, the term tag refers to a specific nucleotide sequence capable of identifying an expressed gene. The 3' end of the tag is defined by the cleavage site of the type III restriction enzyme, and the 5' end of the tag is defined by the cleavage site of another restriction enzyme that is closest to the 3' end of the cDNA.

[0012] "Restriction enzyme" is a general term for endonuclease capable of recognizing a specific sequence of 4 to 8 nucleotides in double-stranded DNA and cleaving it. Restriction enzymes are currently classified into four different groups, called type I, II, III, and IV (Roberts et al. 2003, *Nucleic Acids Res.* 31: 1805-1812). Type III restriction enzymes are complex proteins consisting of methylase and endonuclease subunits and recognizing non-palindromic nucleotide sequences in the target DNA. For endonucleolytic activity, they require the functional cooperation with two copies of the recognition sequence and, moreover, these two copies need to be inversely oriented within the DNA double strand (Muecke et al. 2001, *J. Molec. Biol.* 312: 687-698).

[0013] In the present invention, the above type III restriction enzymes are used for the isolation of tag sequences more than 25 bp in length. Examples of such type III enzymes are disclosed in <http://rebase.neb.com/cgi-bin/azist?re3>. The preferred type III enzymes used in the invention include EcoPI, EcoP15I, and the like.

[0014] In the most preferred embodiment of the invention, the type III restriction enzyme is EcoP15I. The type III restriction enzyme EcoP15I recognizes two unmethylated inversely oriented 5'-CAGCAG-3' sites in the target DNA molecule, and digests 25 to 28 bp apart from the 3'-end of one of the recognition sites. Thus, EcoP15I provides fragments having an overhanging 5' end which is easily blunt-ended using a conventional 3' filling reaction.

[0015] The preferred example of the other enzyme is an enzyme capable of cleaving cDNA into fragments with an average length of 200 bp to 300 bp each, such as:

recognition seq	Enzyme Name (commercially available only)
CATG [^]	NlaIII, Hsp92II,
[^] CATG	FatI
C [^] TAG	Bfa I, MaeI, XspI
A [^] CGT	HpyCH4IV, MaeII,
ACGT [^]	TaiI, TscI
AG [^] CT	AluI
T [^] CGA	TaqI
[^] GATC	BfuCI, Bsp143I, BstENII, DpnII, Kzo9I, MboI, NdeII, Sau3AI
GAT [^] C	BstKTI,
G [^] TAC	Csp6I

The most preferable enzyme is NlaIII.

[0016] The present invention also provides a tag comprising more than 25 nucleotides and capable of identifying an expressed gene, wherein the 3' end of the tag is defined by a cleavage site of the type III restriction enzyme and the 5' end of the tag is defined by the cleavage site of another restriction enzyme that is closest to the 3' end of the cDNA of the expressed gene.

[0017] In the preferred embodiment of the invention, the type III enzyme is EcoP15I and the other restriction enzyme

is an enzyme capable of cleaving cDNA into fragments with an average length of 200 bp to 300 bp each, as described above. The most preferable enzyme is NlaIII.

[0018] The examples of the tag of the invention are shown in Table 1 below. These tags are longer than conventional SAGE tags or LongSAGE tags, and allow more accurate identifications of the corresponding genes.

[0019] The present invention further provides a ditag-oligonucleotide comprising two tags, each of which is derived from a different expressed gene. The ditag-oligonucleotide is produced by the method comprising the following steps:

[0020] 1) synthesizing a cDNA pool from mRNA of expressed genes using a primer comprising oligo-dT and a recognition sequence of the type III restriction enzyme, followed by digestion of the cDNA pool with another restriction enzyme;

[0021] 2) purifying fragments comprising poly A sequence from the above cDNA pool, and ligating the fragments to either Linker-A or Linker-B, both of which comprise the recognition sequence of the type III restriction enzyme;

[0022] 3) digesting the above fragments with the type III restriction enzyme, and ligating the resulting fragment comprising Linker-A to the resulting fragment comprising Linker-B after performing a 3'-filling reaction; and

[0023] 4) digesting the above ligated fragments with the other restriction enzyme of step 1) to cleave off the linker sequence, and thereby obtain the ditag-oligonucleotide.

[0024] As shown in the above steps, a first recognition site of type III restriction enzyme is incorporated into the target cDNA by the RT primer used for the reverse transcription of cDNA from mRNA and a second recognition site of the type III restriction enzyme is incorporated into the target cDNA by the Linker sequence.

[0025] The RT primer is defined by the sequence 5'-N₁₈-25-CAGCAG-T₁₅₋₂₅-3', wherein N₁₈₋₂₅ is an arbitrary nucleotide sequence from 18 to 25 not comprising a sequence 5'-CAGCAG-3' and a sequence 5'-CATG-3'. The 5' end of RT primer may be modified, for example by biotin.

[0026] The type III restriction enzyme of the invention provides fragments having an overhanging 5' end, which can be easily blunt-ended using a conventional 3' filling reaction. Namely, in the above step 3), the fragment comprising Linker-A and the fragment comprising Linker-B can be easily blunt-ended and thereby allow a random association of the fragments without any reduction in tag size.

[0027] In the preferred embodiment of the invention, the type III enzyme is EcoP15I and the other restriction enzyme is an enzyme capable of cleaving cDNA into fragments with an average length of 200 bp to 300 bp each, such as NlaIII.

[0028] In the method of producing the ditag-oligonucleotide of the invention, two linkers (Linker-A and Linker-B) are used. The Linker A and the Linker-B are double-stranded DNA different from each other. One end of the double-stranded linker fragment participating in ligation comprises the recognition sequence of the type III restriction enzyme adjacent to the sequence for the ligation. For example, when the type III restriction enzyme is EcoP15I and the other restriction enzyme is NlaIII, both the linkers comprise the

5'-CAGCAGCATG-3' sequence at the 3' ends of their first strands. The underlined sequence 5'-CAGCAG-3' constitutes one of the recognition sites of EcoP15I, and the 5'-CATG-3' sequence constitutes a 3' overhanging single-stranded region to ligate to the foment generated by NlaIII digestion.

[0029] The other end of the double-stranded linker fragment not participating in ligation may be modified by a labeling reagent such as FITC (Fluorescein Isothiocyanate; the 5'-end of the first strand) and by an amino moiety (the 3'-end of the second strand).

[0030] Thus, the linkers are made by annealing the following first strand of DNA(1) and second strand of DNA(2);

DNA (1) : 5' -N₃₀₋₄₀-CAGCAGCATG-3'
DNA (2) : 3' -N₃₀₋₄₀-GTCGTC-5'

wherein, N₃₀₋₄₀ of DNA(1) and N₃₀₋₄₀ of DNA(2) are arbitrary nucleotide sequences from 30 to 40 bases, which are complementary to each other, and wherein the 5' end of DNA(1) may be labeled and the 3' end of DNA(2) may be amino-modified.

[0031] The present invention further provides a polynucleotide obtained by the ligation of the above ditag-oligonucleotides. Each ditag-oligonucleotide may be cloned and amplified by PCR. The polynucleotide comprises at least 2 ditag-oligonucleotides, and preferably comprises 2 to 200 ditag-oligonucleotides. The ditag-oligonucleotides of the invention are made by a random association of the two tags, and therefore the polynucleotide is also a random concatenation of the tag.

[0032] The present invention further provides a method of gene expression analysis comprising analysis of the nucleotide sequence of the polynucleotide, and quantification of the expression level of an expressed gene based on the number of tags corresponding to the expressed gene included in the polynucleotide.

[0033] For example, the method of invention comprises the following steps:

[0034] 1) synthesizing a cDNA pool from mRNA of expressed genes using an RT primer comprising oligo-dT and a recognition sequence of a type III restriction enzyme, followed by digestion of the cDNA pool with another restriction enzyme;

[0035] 2) purifying fragments comprising poly A sequence from the above cDNA pool, and ligating the fragments to either linker-A or linker-B, both of which comprise the recognition sequence of the type III restriction enzyme;

[0036] 3) digesting the above fragments with the type III restriction enzyme, and ligating the resulting fragment comprising linker-A to the resulting fragment comprising linker-B after performing a 3'-filling reaction;

[0037] 4) digesting the above ligated fragments with the other restriction enzyme of step 1) to cleave off the Linker sequence, and thereby obtain a ditag-oligonucleotide comprising two tags of more than 25 nucleotides and capable of identifying the expressed gene;

[0038] 5) ligating the ditag-oligonucleotides to produce a polynucleotide; and

[0039] 6) analyzing the nucleotide sequence of the above polynucleotide, and quantifying the expression level of an expressed gene based on the number of tags corresponding to the expressed gene included in the polynucleotide.

[0040] In the preferred embodiment of the method, the type III enzyme is EcoP15I and the other restriction enzyme is an enzyme capable of cleaving cDNA into fragments with an average length of 200 bp to 300 bp each, such as NlaIII. Regarding the linkers, the double-stranded DNA as described above may be preferably used.

[0041] The present invention further provides a kit for isolating a tag comprising more than 25 nucleotides and capable of identifying an expressed gene, comprising the following elements:

[0042] a) An RT primer defined by the sequence 5'-N₁₈₋₂₅ not comprising a sequence 5'-CAGCAG-3' and a sequence 5'-CATG-3', and wherein the 5' end of RT primer may be biotinylated.

[0043] b) Linker-A and Linker-B, which are double-stranded DNA different from each other and made by annealing the following first strand of DNA(1) and second strand of DNA(2):

DNA (1) : 5' -N₃₀₋₄₀-CAGCAGCATG-3'
DNA (2) : 3' -N₃₀₋₄₀-GTCGTC-5'

wherein, N₃₀₋₄₀ of (1) and N₃₀₋₄₀ of (2) are arbitrary nucleotide sequences from 30 to 40, which are complementary to each other, and the 5' end of DNA(1) may be labeled and the 3' end of DNA(2) may be amino-modified.

[0044] c) primers capable of hybridizing to the above Linker-A or Linker-B

[0045] The kit may also comprise a type III restriction enzyme such as EcoP15I enzyme and/or another restriction enzyme such as NlaIII. The kit may further comprise, in addition to the aforementioned elements, other elements necessary for carrying out the gene expression analysis of the present invention. Examples include a labeling reagent, a buffer, magnetic beads, or the like.

BRIEF DESCRIPTION OF THE DRAWINGS

[0046] FIG. 1 shows a scheme of the conventional SAGE™ protocol (Velculescu, et al., Science 270:484-487, 1995).

[0047] FIG. 2 shows a schematic procedure for the isolation of 26-bp CDNA SuperSAGE tags using EcoP15I.

[0048] FIG. 3 summarizes the application of SuperSAGE analysis using 26-bp tags as obtained by EcoP15I.

[0049] FIG. 4 shows an example of an electrophoresis of products after EcoP15I digestion (step 6, FIG. 2), visualized by FITC fluorescence. The structure of each fragment is depicted on the right side of the panel. In this example, mRNA molecules derived from rice leaves were used as template for cDNA synthesis.

[0050] FIG. 5 shows an example of an electrophoresis (PAGE) of PCR products from step 9 of FIG. 2. The size of the expected PCR product is ca. 97 bp.

[0051] FIG. 6 shows an example of an electrophoresis (PAGE) of fragments resulting from NlaIII digestion of the PCR products (step 10). The size of the ditag is ca. 52 bp.

[0052] FIG. 7 shows an example of an electrophoresis of concatenated fragments of ditags (step 11).

[0053] FIG. 8 shows an example of an electrophoresis of colony PCR products (step 14).

[0054] FIG. 9 shows an example of the DNA sequence contained in the cloned concatemer (step 15).

[0055] FIG. 10 shows the results of RT-PCR using RNAs isolated from *Magnaporthe grisea*-infected rice leaves using 26-bp tag sequences as PCR primer.

[0056] FIG. 11 shows the results of RT-PCR in *Nicotiana benthamiana* that were either treated with INF1 elicitor protein from *Phytophthora infestans* or water as a control.

[0057] FIG. 12 shows the RT-PCR kinetic study of gene expression of four genes that were identified by Super-SAGE.

PREFERRED EMBODIMENTS OF THE PRESENT INVENTION

[0058] Referring to FIG. 2, the preferred embodiment of the present invention using EcoP15I as a type III restriction enzyme is described hereinafter.

[0059] The type III restriction-modification enzyme EcoP15I recognizes two unmethylated inversely oriented 5'-CAGCAG-3' sites in the target DNA molecule, and digests 25 to 28 bp apart from the 3'-end of one of the recognition sites. The invention refers to all potential applications of the EcoP15I enzyme for the isolation of 25-to 28-bp tag sequences from a defined position of cDNAs.

[0060] Double-stranded cDNA is synthesized from mRNA using a biotinylated oligo-dT-anchor primer (hereinafter referred as "RT primer" or reverse transcription primer). This RT primer comprises an arbitrary nucleotide sequence from 18 to 25 bases and the 5'-CAGCAG-3' sequence followed by an oligo-dT sequence from 15 to 25 bases. The 5'-CAGCAG-3' sequence included in the RT primer constitutes one of the recognition sites of the EcoP15I (FIG. 2, step 1). For example, the RT primer comprising a 22-nucleotide sequence and the 5'-CAGCAG-3' sequence followed by 19-dT sequence is:

(SEQ ID NO:1)

5'-CTGATCTAGAGGTACCGGATCC**CAGCAG**TTTTTTTTTTTTTTT
T-3'.

[0061] Synthesized CDNA is digested by restriction endonuclease NlaIII, which recognizes the sequence motif 5'-CATG-3'. Only the digested fragments comprising RT primer sequences (biotin-labeled) are captured by streptavidin-coated magnetic beads (FIG. 2, steps 2 and 3).

[0062] A double-stranded linker fragment (46 bp) is ligated to the ends of the CDNA fragment (comprising a

poly A sequence) captured by magnetic beads. One end of this linker fragment participating in ligation comprises the 5'-CAGCAG-3' sequence adjacent to the 5'-CATG-3' sequence in the first strand (FIG. 2, step 4). The 5'-CAGCAG-3' sequence constitutes one of the recognition sites of EcoP15I, and the 5'-CATG-3' sequence constitutes a 3' overhanging single-stranded region to be ligated to the cohesive end of the fragments generated by NlaIII digestion. The other end of the double-stranded linker fragment not participating in ligation is modified by FITC (Fluorescein Isothiocyanate; the 5'-end of the first strand) and an amino moiety (the 3'-end of the second strand).

[0063] In the present invention, two linkers are used. For example, Linker-A is made by annealing the following two oligonucleotides:

(SEQ ID NO:2)

FITC-5'-TTTGGATTGCTGGTGCACTACAAGCTTAATAC**CAGCAG**
CATG-3'
and
5'-**CTGCTGTATTAAGCCTAGTTGTACTGCACCAGCAATCCAAA**-3'-
NH₂.

[0064] Linker-B is made by annealing the following two oligonucleotides:

(SEQ ID NO:4)

FITC5'-TTTCTGCTCGAATTCAAGCTTCTAACGATGTACG**CAGCAG**CAT
G-3'
and
5'-**CTGCTGCGTACATCGTTAGAAGCTTGAATTCGAGCAGAAA**-3'-
-NH₂.

[0065] The cDNA pool is divided into two halves, with one half ligated to the Linker-A and the other half to the Linker-B, resulting in the "Linker-A ligated cDNAs" and the "Linker-B ligated cDNAs".

[0066] For both Linker-A ligated cDNAs and Linker-B ligated cDNAs, DNA fragments bound to the beads are digested with EcoP15I (FIG. 2, step 5). EcoP15I recognizes a pair of inversely oriented motifs of the sequence 5'-CAGCAG-3', and cleaves 25 to 28 bp apart from the 3'-end of one of the recognition sites. After digestion, two fragments are released from the beads. One is the fragment comprising the linker and the 27- or 28-bp tag fragment (with a total size of 69 or 70 bp), and the other one is a fragment of variable size located in the middle of the double-stranded cDNA fragments. The fragments comprising the poly-A sequence remain bound to the magnetic beads, and do not participate in the following procedure.

[0067] The 69- or 70-bp fragment comprising the linker and the 27- or 28-bp tag sequence are visualized by FITC fluorescence under UV radiation, and easily isolated from a polyacrylamide gel by gel excision.

[0068] EcoP15I provides fragments having an overhanging 5' end, which are easily blunt-ended by the conventional

3'-filling reaction, thereby allowing the random association of the fragments. Therefore, the 69- or 70-bp fragments (linker-tag fragments) originating from Linker-A-ligated cDNAs and Linker-B-ligated cDNAs, respectively, are each blunt-ended by 3'-filling reaction and ligated to each other to form ditags by random association of two tags. The 3'-ends of linker fragments are blocked by an amino-modification, so that ligation occurs only between cDNA tag sequences sides that are blunt-ended (FIG. 2, steps 6, 7).

[0069] Resulting ditag molecules are amplified by PCR (FIG. 2, step 9). Examples of PCR primers designed from the linker sequences are shown below:

Ditag primer 1E:
biotin-5'-CAACTAGGCTTAATACAGCAGCA-3' (SEQ ID NO:6)

Ditag primer 2E:
biotin-5'-CTAACGATGTACGACAGCAGCA-3' (SEQ ID NO:7)

The expected size of the PCR product obtained by PCR using the above primers is ca. 97 bp.

[0070] The ca. 97 bp PCR product is digested with NlaIII (FIG. 2, step 10, thereby releasing ca. 52-bp ditag fragments. These fragments are recovered from the gel, and purified.

[0071] Ditag fragments are concatenated by a ligation reaction (FIG. 2, step 11). Concatemers are separated by agarose gel electrophoresis. Fragments larger than 500 bp are eluted from gel and recovered.

[0072] Size-separated concatemer fragments are ligated to an appropriate plasmid vector that is predigested with SphI and treated with calf intestine phosphatase (FIG. 2; step 12), and the plasmids transformed into *E. coli* (FIG. 2; step 13).

[0073] The insert fragments of the plasmids are PCR amplified (FIG. 2; step 14).

[0074] The PCR products are directly sequenced (FIG. 2; step 15). A series of ca. 44 bp ditag sequences are flanked by the NlaIII recognition sequence CATG. This ca. 52 (44+8)-bp sequence information provides two 26- to 28-bp tag sequences isolated from a defined position of each cDNA.

[0075] After counting the number of 26-bp tags in a sample, it is possible to obtain the gene expression profiles as described in the original SAGE™ protocol. Since the ditags of the invention are formed by the random association of blunt-ended tags, the result faithfully reflects the expression of each gene.

[0076] The 26-bp tag sequence contains sufficient information to uniquely identify the gene from which the tag was derived. With the information content in the 26-bp DNA sequence, in silico identification of the corresponding gene is facilitated. Even a BLAST search of a 26-bp tag sequence against the entire body of Genbank sequences will show the correct match for the gene from which the tag originated (FIG. 3).

[0077] In case the described method is applied for organisms for which no DNA sequence data is available, the 26-bp tag sequence can directly be used as the PCR primer for 3'-RACE to recover the 3'-region of the cDNA. Such cDNA sequence can be used for a BLAST search to identify the gene (FIG. 3).

[0078] The 3'-RACE with a 26-bp tag sequence can be directly performed as RT-PCR to quantify the amount of messages for the verification of the gene expression difference between the samples as revealed by the SuperSAGE (FIG. 3).

[0079] The 26-bp tag sequence is longer than the minimum size (21 bp) of DNA sequence necessary for triggering "RNA interference" (RNAi) (Elbashir et al. Nature 411: 494-498, 2001). Therefore, double-stranded RNA comprising the tag sequence could be immediately used for the functional analysis to knock out the gene corresponding to the tag. This means that gene expression analysis as performed by SuperSAGE could be directly connected to gene function analysis with the 26-bp tag isolation.

[0080] For application in plant species, the 3'-RACE fragment as described above could be cloned into a plant virus vector, and used for "virus-induced gene silencing (VIGS)" (Baulcombe, Curr. Opin. Plant Biol. 2: 109-113, 1999), and thus the described SuperSAGE method connects the gene expression analysis to gene function analysis in plants as well.

EXAMPLES

[0081] The present invention is described in greater detail with reference to the following examples, although the scope of the present invention is not limited to these examples.

Example 1

[0082] As a proof of principle, 26- and 27-bp tag sequences were isolated from leaves of a lesion-mimic mutant IB2020 of rice (*Oryza sativa* cv. Kakehashi) by the method described above.

1) Preparation of mRNA

[0083] Total RNA was isolated from leaf blades of rice by a conventional RNA isolation method. From this RNA, 5 µg of mRNA were isolated using an "mRNA Purification Kit" (Amersham Pharmacia). The mRNA was dissolved in 29 µl of DEPC water, and used as source material.

2) cDNA Synthesis Using oligo-dT Primer

[0084] This mRNA was reverse-transcribed using a "cDNA Synthesis System" (Invitrogen) to generate single-stranded cDNA using the following reverse transcription-primer comprising the 5'-CAGCAG-3' motif that is a recognition sequence of the enzyme EcoP15I.

[0085] Reverse Transcription-Primer:

(SEQ ID NO:1)
5'-CTGATCTAGAGGTACCGGATCCAGCAGTTTTTTTTTTTTTTTT
T-3'

The product was converted to double-stranded cDNA using the same kit ethanol precipitated, and dissolved in 20 µL of LoTE buffer (3 mM Tris-HCl pH 7.5, 0.2 mM EDTA).

3) NlaIII Digestion

[0086] Resulting double-stranded cDNA (20 µL) was digested in 200 µL reaction solution comprising 50 units of NlaIII (New England BioLabs; NEB) in 1xNEB Buffer 4

(NEB) containing 0.1 mg/ml BSA at 37° C. for 90 min. After digestion, cDNA was extracted with TE-equilibrated Phenol/Chloroform/Isoamylalcohol (25:24:1; pH 8.0), ethanol precipitated, and dissolved in 20 µl LoTE buffer.

[0087] In each of two Eppendorf tubes, 1 ml streptavidin magnetic beads suspension (Streptavidin Magnesphere Paramagnetic particle, Promega) was transferred. Magnetic beads were washed once with 200 µl 1×B&W solution (5 mM Tris-HCl pH 7.5, 0.5 mM EDTA, 1M NaCl) using a magnetic separation stand (Magnesphere Technology Magnetic Separation Stand). After washing, 100 µl 1×B&W buffer and 10 µl cDNA obtained from the above step and 90 µl water were added to each tube, mixed, and incubated at room temperature for 30 min. The cDNA bound by magnetic beads was washed three times with 1×B&W buffer and three times with LoTE buffer.

4) Linker Ligation

[0088] The following oligonucleotides were synthesized commercially (Qiagen).

Linker-A1: (SEQ ID NO:2)
FITC-5'-TTTGGATTGCTGGTGCAGTACAACCTAGGCTTAATACAGCAG

CATG-3'

Linker-A2: (SEQ ID NO:3)
5'-CTGCTGTATTAAGCCTAGTTGTACTGCACCAGCAAATCCAAA-3'-

NH₂

Linker-B1: (SEQ ID NO:4)
FITC5'-TTTCTGCTCGAATTCAAGCTTCTAACGATGTACGCAGCAGCA

TG-3'

Linker-B2: (SEQ ID NO:5)
5'-CTGCTCGGTACATCGTTAGAAGCTTGAATTCGAGCAGAAA-3'-

NH₂

The 5'-termini of Linker-A2 and Linker-B2 were phosphorylated by T4 polynucleotide kinase (NEB). Linker-A was prepared by annealing Linker-A1 and phosphorylated Linker-A2, and Linker-B by annealing Linker-B1 and phosphorylated Linker-B2. Both Linker-A and Linker-B harbor the EcoP15I recognition sequence (5'-CAGCAG-3').

[0089] To each of the two tubes containing cDNA bound to magnetic beads, 17 µl LoTE, 3 µl 5× ligase buffer (Invitrogen) and 1 µg either of Linker-A or Linker-B were added. After mixing, tubes were incubated at 50° C. for 2 min and left at room temperature for 15 min. Subsequently, 2 µl T4 DNA ligase (5 units/µl, Invitrogen) was added to the tube and incubated at 16° C. for 90 min. After linker ligation, the beads were washed 4 times with 1×B&W, 3 times with LoTE buffer and once with sterile distilled water.

5) EcoP15I Digestion

[0090] Linker-ligated cDNA on the magnetic beads was digested with 10 units EcoP15I in 100 µl reaction mixture (10 mM Tris-HCl pH 8.0, 10 mM KCl, 10 mM MgCl₂, 0.1 mM EDTA, 0.1 mM DTT, 5 µg/ml BSA, 2 mM ATP). Tubes were incubated at 37° C. for 90 min.

6) Purification of Linker-Tag Fragments

[0091] After EcoP15I digestion, the tubes were placed on a magnetic stand. After removal of the biotinylated outermost 3'-terminal fragment by streptavidin coated magnetic beads, the unbound fraction containing the linker-tag fragments was collected, and transferred to a new tube. Collected solution was extracted by Phenol/Chloroform/Isoamylalcohol (25:24:1; pH 8.0), ethanol precipitated, dissolved in 10 µl LoTE buffer, and separated by 8% polyacrylamide gel electrophoresis. In adjacent lanes, size marker (20 bp ladder, TAKARA) prestained with SYBR green (FMC) was loaded. After electrophoresis, the gel was placed on an UV illuminator, and the linker-tag fragment of ca. 69 bp in size was visualized by its FITC-mediated fluorescence (FIG. 4). Two additional fragments presumably having originated from linker-linker ligate (ca. 90 bp) and single linker fragments (46 bp) were also visualized. In case the FITC-fluorescence gave too weak signal, the gel could be stained with SYBR green (FMC) to visualize the linker-tag fragment. The ca. 69-bp linker-tag fragment was cut out from the gel and placed into a 0.5 ml tube with a pinhole in the bottom made by a syringe needle. The 0.5 ml tube was placed inside a 2 ml tube, and centrifuged at 15000 rpm for 2 min. To the small gel pieces collected at the bottom of the 2 ml tube, LoTE buffer (300 µl) was added, and they were incubated at 37° C. for 2 hrs, followed by incubation at 65° C. for 15 min. The gel suspension was transferred to a SpinX column (Coaster) and centrifuged at 15000 rpm for 2 min. Recovered solution was extracted once by Phenol/Chloroform/Isoamylalcohol (25:24:1; pH 8.0), ethanol precipitated and dissolved in 8 µl LoTE buffer.

7) Blunting of Tag Fragment

[0092] The 5'-protrusion of the linker-tag fragments was filled-in by DNA polymerase using a kit (Blunting High; TOYOBO). To the solution containing the linker-tag fragment (8 µl) 1 µl reaction buffer (KOD buffer, TOYOBO) and 1 µl KOD polymerase (TOYOBO) were added, and it was incubated at 72° C. for 2 min

8) Ligation for Ditag Formation

[0093] Linker-tag fragments that had originated from Linker-A and Linker-B were ligated to form the ditag fragments. Equal volumes (2 µl) of blunt-ended Linker-A-tag and Linker-B-tag solutions were mixed, and 6 µl LoTE and 10 µl ligation mixture (Ligation High, TOYOBO) were added. Ligation solution was incubated at 16° C. from 4 hrs to overnight

9) Ditag PCR

[0094] Resulting ditag solution was diluted five- and ten-fold, and used as template for ditag PCR. The PCR reaction was made in 288 (=96×3) 0.2 ml tubes each containing 50 µL solution containing 1 µL diluted ditag template, 3 units Taq polymerase (AmpliTaq Gold; Applied Biosystems), 1× AmpliTaq Gold buffer and the PCR primers. The 5'-ends of the PCR primers were biotinylated as follows (commercially synthesized by Qiagen).

Ditag primer 1E:
biotin-5'-CAACTAGGCTTAATACAGCAGCA-3' (SEQ ID NO:6)

Ditag primer 2E:
biotin-5'-CTAACGATGTACGCAGCAGCA-3' (SEQ ID NO:7)

PCR consisted of initial denaturation at 95° C. for 12 min followed by 27-29 cycles of 94° C. for 40 sec and 60° C. for 40 sec. Expected size of the amplified ditag fragments was ca. 97 bp (FIG. 5).

10) Purification of ditag PCR products

[0095] Ditag PCR product (about 300 tubes) was bulked in 10 ml plastic tubes. After Phenol/Chloroform/Isoamylalcohol (25:24:1; pH 8.0) extraction and ethanol precipitation, it was dissolved in 100 µl LoTE buffer. This PCR product was run on a 1.5% low-melting Agarose (SeaPlaque, FMC) gel, and the ca. 97 bp fragment was cut out from gel, which was purified by a Qiagen Gel extraction kit (Qiagen).

11) Linker removal by digestion with NlaIII

[0096] The purified ca 97 bp fragment eluted in 121 µl LoTE buffer was digested with 120 units NlaIII (NEB) in 1×NEB buffer 4 containing 0.1 mg/ml BSA (NEB). After confirmation of digestion by gel electrophoresis (three fragments of 52 bp, 22 bp and 23 bp in size were visualized, FIG. 6), digestion solution was treated by streptavidin magnetic beads at room temperature for 30 min for the removal of linker fragments. After the magnetic beads in the tube were collected by magnetic stand, the supernatant was transferred to a new tube, and the DNA extracted with Phenol/Chloroform/Isoamylalcohol (25:24:1; pH 8.0), ethanol precipitated and dissolved in 10 µl LoTE buffer.

12) Purification of 52 bp ditag fragment

[0097] Recovered DNA was electrophoresed in 12% polyacrylamide gel. To visualize the fragment, the gel was stained with SYBR green (FMC). A fragment of ca. 52 bp in size was cut out from the gel on a UV transilluminator. DNA was eluted from the gel as described above. DNA solution eluted from the gel was treated by streptavidin magnetic beads at room temperature for 30 min. Supernatant was collected, and extracted by Phenol/Chloroform/Isoamylalcohol (25:24:1; pH 8.0), ethanol precipitated and dissolved in 7 µl LoTE buffer.

13) Concatemer formation

[0098] For concatenation of ditag fragments, 7 µl of the ditag solution, 2 µl 5×ligase buffer, and 5 units T4 ligase (Invitrogen) were mixed and incubated at 16° C. for 14 hrs. The ligation solution was incubated at 65° C. for 10 min., and loaded on 1% agarose gel (FIG. 7). DNA fragments of more than 500 bp in size were cut out from gel and eluted. Purified concatemers were dissolved in 6 µl LoTE buffer.

14) Vector cloning

[0099] Five micrograms of cloning vector (pGEM3Z, Promega) was digested with SphI, and treated with calf intestine alkaline phosphates (CIAP). For cloning the con-

catemers, this digested pGEM3Z vector and the ditag concatemers were ligated using T4 ligase (Invitrogen). After 4 hrs ligation, reaction solution was extracted with Phenol/Chloroform/Isoamylalcohol (25:24:1), and ethanol precipitated. The pellet was washed 4 times with 70% ethanol to completely remove salt and dissolved in 10 µl sterile distilled water.

15) Transformation

[0100] Electrocompetent *E. coli* cells (DH10B, Invitrogen) were used for the cloning of plasmids containing the ditag concatemers. In a tube, 40 µl competent cell suspension and 1 µl purified ligated DNA were mixed on ice, and transferred into an electroporation cuvette (0.1 cm gap; BioRad). Conditions for electroporation were as recommended by the manufacturer of the competent cells (2.5 kV, 25 µFD, 100ω). After electroporation, 1 ml SOC medium (Invitrogen) was added, and the solution was incubated at 37° C. for 1 hr. The resulting *E. coli* suspension was plated on an LB medium containing 100 µg/ml Ampicillin, 20 µg/ml X-gal and 0.1 mM IPTG. Plates were incubated at 37° C. for 14 hrs.

16) Colony PCR and Sequencing

[0101] White colonies developed on the plates were picked up using toothpicks, and resuspended in the PCR mixture containing 1× PCR buffer, 0.4 mM dNTP, 0.05 unit/µl Taq polymerase (TAKARA), 2 pmols/µl M13-20 primer and 2 pmols/µl M13RV primer. Cloned fragments in the plasmid were amplified by PCR and the fragment size checked by agarose gel electrophoresis (FIG. 8). The amplified PCR fragments were purified using a PCR purification kit (Qiagen).

17) Sequencing of Concatemers

[0102] The purified PCR fragments (concatemers) were sequenced with the Big dye terminator (Applied Biosystems) and M13-20 primer. After the sequencing reaction, the DNA was purified by the "Montage SEQ96 Sequencing Reaction Cleanup Kit" (Milipore). DNA sequences were analyzed by the RISA384 capilla DNA sequencer (Shimadzu). An example of the DNA sequences of cloned fragments is shown in FIG. 9. A series of 52- to 54 bp ditags comprising two 26- or 27-bp fragments are delimited by CATG NlaIII sites.

18) Data analysis

[0103] From the DNA sequences of concatemers, 26-bp tag sequences were extracted and the number of each tag counted using SAGE™ 2000 software, supplied by Johns Hopkins University. After sequencing several clones, a gene expression profiling as shown in Table 1 was performed.

TABLE 1

An example of SuperSAGE result obtained from rice leaves. Tag sequences are represented by excluding the shared NlaIII site (CATG).			
SuperSAGE tag	corresponding SAGE tag	No of tags	Corresponding genes
GATCCGTCTCTCTGGGAGGAAT	GATCCGTCTC	5	TC83196 Thiazole biosynthetic enzyme
TTGTAATACTCCATCAAGAGT	TTGTAATACT	3	TC82944 catalase

TABLE 1-continued

An example of SuperSAGE result obtained from rice leaves.
 Tag sequences are represented by excluding the shared NlaIII site (CATG).

SuperSAGE tag	corresponding SAGE tag	No of tags	Corresponding genes
CGAAATCGATTCCGAGTCTCT	CGAAATCGAT	2	thioredoxin h
ATGGCCTGAGGAAGTGGCTCGC	ATGGCCTGAG	2	TC89949 triosephosphate isomerase
ATGATGATATACTACACTTGAT	ATGATGATAT	2	TC89920 photosystem II 10 kDa polypeptide
TTCGGCTTCTTCGTCCAGGCCA	TTCGGCTTCT	2	BI809989 chlorophyll a/b-binding protein
TTGTAACTCGTTATATCCTCA	TTGTAACTC	2	TC84234 homeobox gene
CTCAAGATGATCGAGGACTACC	CTCAAGATGTA	2	TC90193 PBZI
TATATTTATCCATAATATACTC	TATATTTATC	2	TC83801
TAATGGTACATATCTCCTTGTT	TAATGGTACA	2	TC83132
ACGATTGGTGAATACCCTTGGA	ACGATTGGTG	1	AU082990
TTGTGATTCAACTCTAGTAG	TTGTGATTCA	1	N
TACATAAATGGAAAAAGAGAGG	TACATAAATG	1	genomic DNA AP004997
TTTGACAAGACATTTTCAGTAT	TTTGACAAGA	1	TC90371 GF14-d protein
GAATCAATACTGTAACATATTAT	GAATCAATAC	1	TC83400 thioredoxin M
TTCATATGAAATAAAATATGA	TTCATATGAA	1	BI806753
GGGGGCGGCAAGCCGACGATCG	GGGGGCGGCA	1	AU070324
TATGTATGTACCTTAATTGTGT	TATGTATGTA	1	TC82787 chlorophyll a-b binding protein
TGTTACTAGCTGCTACTGTCTA	TGTTACTAGC	1	D43299
GCTCTTTCTGTTTGAGGGCCAT	GCTCTTTCTG	1	TC83133
GCTGTGTTGATCTAAACTGTT	GCTGTGTTGT	1	TC90306
TAAACAAACCTTCTAAGAGACA	TAAACAAACC	1	TC90893
TCGTGGGGTATCTAGGGCTGGA	TCGTGGGGTA	1	N
AGCATTGTTTTACACGTAATGC	AGCATTGTTT	1	genomic DNA AP003249
AAGCTACTTGCTTCCCTTGCTC	AAGCTACTTG	1	AA751992
GGTTTCAGCTTGTTGACTAAT	GGTTTCAGCT	1	TC83067 acidic ribosomal protein
ATGTAGATCGATGGATGTACCA	ATGTAGATCG	1	genomic DNA OSJN00066
CATATGTTGATCAATCTGGAGG	CATATGTTGA	1	AP003685
TAACTTACTCTTGGTTAATTAT	TAACTTACTC	1	TC83288
TGTAACCTCACTGGATTGGAGTG	TGTAACCTCAC	1	TC88697
GTAATTGCTCTGGGTGATTCT	GTAATTGTCC	1	N
CTGGTATTCTGCAACGTTACAT	CTGGTATTCT	1	N
GGAAAAGCTACTGACTGGTAAT	GGAAAAGCTA	1	TC91674 Adenylosuccinate synthetase
GGACAATGTCACTTGTTTCACA	GGACAATGTC	1	AU071217
ATAATTATTGCTGGATGATCAA	ATAATTATTG	1	genomic DNA OSJN00139
TTCATCAGCATATTTTAAATA	TTCATCAGCA	1	TC84963
GATCATTGCTTGATGGTAAAGA	GATCATTGCT	1	TC83935 ADP-ribosylation factor

TABLE 1-continued

An example of SuperSAGE result obtained from rice leaves.
Tag sequences are represented by excluding the shared NlaIII site (CATG).

SuperSAGE tag	corresponding SAGE tag	No of tags	Corresponding genes
CGGGTGCTCCTACATCTCTTCT	CGGGTGCTCC	1	AU070302
GTCCGCGCCCCCTGCGTCGTCG	GTCCGCGCCC	1	TC83870
TATCAATGTATTTTATCTGTGTA	TATCAATGTA	1	TC82917 abscisic acid- and stress-inducible protein
TAATAATCTGTAACTGAAGAC	TAATAATCTG	1	TC90227 catalase
TAGAGTTAGAGATATTTGAGCT	TAGAGTTAGA	1	N
CGATTTTGAGTAAATACGCTA	CGATTTTGAG	1	TC92829
GGCCGCCTCCCGTGTGTCGTG	GGCCGCCTCC	1	TC90664 l-asparaginase
ATATATCACAAATGTCTGATCCA	ATATATCACA	1	N
GTCCCAGCTTACTGCAAGATCA	GTCCCAGCTT	1	TC82994 carbonate dehydratase
GTCGCCTCTCTGCTCGTGCCCC	GTCGCCTCTC	1	TC89722
CGAGAATGCTAATGGGCGATCA	CGAGAATGCT	1	genomic DNA AC118672
GCGGCGGCGATGGTCCCTCCCG	GCGGCGGCGA	1	TC83113
AGATACTGTACAGGATTGAATG	AGATACTGTA	1	TC86544
GCCGCCACCTCGCTGTGCGCGC	GCCGCCACCT	1	TC90110 Photosystem I reaction center subunit V
CCTAACTTGCTGTAAGCACATT	CCTAACTTCC	1	TC90578
GCAAGTTACCAATGGAAAATA	GCAAGTTACC	1	N
ATATATATATAAAAAGGAATAG	ATATATATAT	1	N
CAGGCCGTCGCGCTCGACGTGC	CAGGCCGTCG	1	genomic DNA AP005284
AACCAGGATTCACCAGTTCTGG	AACCAGGATT	1	TC89467
TATATGTTTTCTAGTTTTCTCT	TATATGTTTT	1	genmic DNA AP005056
TAACAAGCAGATTAGAAAGCTG	TAACAAGCAG	1	AU089764
CAGCAATACTCTGTAATATTAT	CAGCAATACT	1	TC84453
AGTGACTGTTGTTGTAGAGAAC	AGTGACTGTT	1	genomic DNA AC090874
GCTTGAGCCCTGGGGCCCTGGC	GCTTGAGCCC	1	genomic DNA AP003408
AAGTGTTATACTGTACACTGAT	AAGTGTTATA	1	N
TGTAACATAATGAAATGGTGTG	TGTAACATAA	1	TC84198 small GTP-binding protein
GAGAAAATTAACTGAAAAAAA	GAGAAAATTA	1	N
TTTGATGCTTGACAGGCAACAT	TTTGATCCTT	1	TC84043 remorin
GCTATTGCAACTGTGGTGACTG	GCTATTGCAA	1	TC84281
TAACAAGCGAGGTGCTTGTGTC	TAACAAGCGA	1	TC89910
CCAATACTGGTGAAAAAATAA	CCAATACTGG	1	N
GAACCTTTGGGATGTAAGCACTG	GAACCTTTGGG	1	genomic DNA AC103891
TAGCAATGCCAGGGATTGTAT	TAGCAATGCC	1	TC85883
GGATATTGGAATGGGAGTATAA	GGATATTGGA	1	genomic DNA AC135258
CTCGACTTCGCCGCGAGCACG	CTCGACTTCG	1	TC86797 sinapyl alcohol dehydrogenase

TABLE 1-continued

An example of SuperSAGE result obtained from rice leaves.
Tag sequences are represented by excluding the shared NlaIII site (CATG).

SuperSAGE tag	corresponding SAGE tag	No of tags	Corresponding genes
GCACCCACGAGAGAGGGGGCTC	GCACCCACGA	1	TC91365
GACGAGGACCCCCGGCGGGTG	GACGAGGACG	1	TC82846 putative HSP70
TGTTAGTATTATGTAACCTCTGT	TGTTAGTATT	1	TC87007
GCCGCCGAGCCGGAGATACCGG	GCCGCCGAGC	1	TC89744 glyceraldehyde-3-phosphate dehydrogenase
GGTATGCTTCCATTGTTCAGCAG	GGTATGCTTC	1	N
TAATTTTAATCATCTGTTCAGGC	TAATTTTAAT	1	TC83170 Photosystem I reaction center subunit II
GATATGGACGAGTGTGTTATTT	GATATGGACG	1	TC84236
TGTTAAACTTGCCATTCTGATA	TGTTAAACTT	1	TC90900 Elongation factor 1-gamma
TTGTCAAGGGCCAGTTGGATGT	TTGTCAAGGG	1	AU031603
GCACCTGACACCGACACTGAAG	GCACCTGACA	1	AT003457
GGATGCCACTGGATCGAAAAGA	GGATGCCACT	1	TC85434
GATAGCAAGTAGTAGCCTGTTT	GATAGCAAGT	1	TC84953
CAATCCTTGATCCGAACCTGTGC	CAATCCTTGA	1	TC85407
ACCCGAGTGTGCGTCTTCAGTT	ACCCGAGTGT	1	TC90603 malate dehydrogenase
GGAGCTTGACATCAATGGCAGA	GGAGCTTGAC	1	TC92018
CAGCGGCGGCTTAGGCGCGTGG	CAGCGGCGGC	1	TC84795
GAACCAAAATTTTCAGAGACCC	GAACCAAAAT	1	TC84932
CCTCACGGCATCTTAACCTAGAA	CCTCACGGCA	1	TC83665
ACCATATTCTTGTGTACTGCC	ACCATATTCT	1	TC91249
TTCTTGATAAGGGATCGATTAG	TTCTTGATAA	1	genomic DNA AL606615
TACTACTACCTTGTAACCTTTT	TACTACTACC	1	BE228969
GTTGAGCAGGAGCTGCCTTCTG	GTTGAGCAGG	1	TC90020 S-adenosylmethionine decarboxylase
TTCGGCTGCACCGATGCCACCC	TTCGGCTGCA	1	TC89652 small subunit of ribulose-1,5-bisphosphate carboxylase
GCCGGTGGGCAGGAGCAAGCGG	GCCGGTGGGC	1	TC83622
TAGTAGGAAGTTGACATCTTAC	TAGTAGGAAG	1	TC91942 heat shock protein 70
AGGGCCGGCGGCAAGCGGCGG	AGGGCCGGCG	1	NP340063
CAGGCAGACAGATCCGTGTGTA	CAGGCAGACA	1	TC92072

[0104] Thus, 26- or 27-bp sequences adjacent to the NlaIII sites of cDNAs were successfully isolated from rice leaves. The identification tags comprising 26- or 27-bp sequences can be obtained from any cDNA by applying the experimental procedure described herein. This protocol based on the isolation of tags comprising 26- or 27-bp sequences represents a substantial qualitative improvement over the most advanced SAGE™ procedure (known as “Long-SAGE”) using tags comprising 18- to 21-bp sequences.

[0105] In the SuperSAGE method, EcoP15I enzyme generates 5'-overhanging ends in the tags that are readily blunt-ended by DNA polymerase. Therefore, ditag formation is made by random association of the blunt-ended tags, thereby ensuring that the number of tags faithfully represents the expression of the genes corresponding to the tags. This property is not available when ditags are formed only between the 21-bp tags comprising compatible 2-bp 3'-overhanging ends in LongSAGE™ protocol. To secure the random association of tags in LongSAGE™, the tags should be blunt-ended by removing the 3'-overhanging region. This results in a 2-bp reduction of tag size and thereby reduces the information content of the tags. Thus, the SuperSAGE method described herein is advantageous over the LongSAGE™ method from the viewpoint of the larger information content of the tags and the more faithful representation of gene expression profiles.

Example 2

[0106] To demonstrate that the 26-bp tag size of SuperSAGE allows a highly reliable annotation of the tag to the gene, the following experiment was conducted.

[0107] Thirty 26-bp tags were randomly selected from Table 1. These DNA sequences were truncated from the 3'-ends so that the tag sizes became 20, 18 and 15 bp, respectively. The tag size of 15 bp corresponds to that used in the conventional SAGE™. The 18-bp or 20-bp tags were extracted from the tag of LongSAGE™, when the linker-tag fragments were ligated to each other with and without end-blunting, respectively. Using each of the tags of different sizes, a BLAST search was conducted from the entire body of DNA sequences deposited in Genbank representing DNA sequences from more than 130,000 species. The number of species that contained DNA sequences showing a perfect match to a tag of a given size was counted, and the average and maximum numbers of species were obtained across the 30 tag sequences. The search result are summarized in Table 2.

TABLE 2

Summary of BLAST search of 30 rice SAGE tags for the entire body of Genbank data:				
	Tag size (bp)			
	26	20	18	15
Average number of species with DNA sequence perfectly matching the tag	1.065	1.161	1.840	4.968
Maximum number of species with DNA sequence perfectly matching the tag	2	4	7	9
Number of tags for which DNA sequences of more than two species showed perfect matches	2	3	10	30

[0108] A number of DNA sequences showed a perfect match to a tag sequence, and an increase in tag size reduced the ambiguity of annotation of a tag to a gene.

[0109] The conventional SAGE™ tag (15 bp) matched DNA sequences of more than 4 species on average, and with a maximum of 9 species. All of the 30 tags were correlated with two or more species (Table 2). The 18-bp tags matches 1.8 species on average, with a maximum of 7 species. Ten tags out of 30 were correlated with two or more species. The 20-bp tags matched 1.16 species on average, with a maximum of 4 species. Only 3 tags out of 30 were correlated with more of the two species, indicating a great improvement over the original SAGE™ tag length (15 bp). However, note that 20-bp tag could be extracted only when the linker-tags were ligated without blunting the ends, so that the final results of this method do not necessarily represent accurate gene expression. The 26-bp tags of the SuperSAGE method matched 1.06 species on average, with a maximum of only 2 species. As few as 2 tags out of 30 were correlated with the DNA sequences of more than 2 species. These results clearly show that the information content in the 26-bp DNA sequence provides a great improvement in efficiency of gene annotation of the tags.

[0110] The 26 bp tags matched DNA sequences of only one species on average, and in most cases matched a single gene of the particular species. Thus, the annotation of the tag sequence can be carried out almost perfectly. Tag annotation in SuperSAGE can be performed against EST sequence database as well as against whole genome sequences.

[0111] The high information content of the 26bp tag in SuperSAGE allows the simultaneous gene expression analysis of two organisms. As an example, we applied the described method to study the gene expression profiles of rice plants infected with blast disease caused by the fungus *Magnaporthe grisea*. After isolating a total of 12,119 tags from blast-infected rice leaves, each tag was annotated by BLAST search for all the genome sequences of rice and *M. grisea*. As expected, the majority of the tags were annotated to rice genes (Table 3), while 74 tags did not match rice sequences but matched blast sequences (Table 4).

TABLE 3

The 10 most abundantly expressed genes in blast-infected rice leaves as revealed by Super SAGE		
Tag*	Count	Accession No.: Genes
TTCGGCTTCTTCGTCCAGGCCA	122	D00641: chlorophyll a/b binding protein
GATCCGTCTCTCTGGGAGGAAT	116	AU172529: thiazole biosynthetic enzyme
GCGACGCATCGCCTTCAGCTAA	114	X13909: chlorophyll a/b binding protein
TGGTGGCTTAGCTCTACGTGTA	111	AU174449: glycine rich protein
TCCGACAAGTGC GGCAACTGCG	94	AF001396: metallothionein
TTGTAATACTCCATCAAGAGT	86	D29966: catalase

TABLE 3-continued

The 10 most abundantly expressed genes in blast-infected rice leaves as revealed by Super SAGE		
Tag*	Count	Accession No.: Genes
AATTGAGTTCGCTTTGGTTATG	78	AF010579: glycine rich protein
ATGATGATATACTACACTTGAT	58	BE230408: photo-system II 10 KDa protein
GCGTCCACGCTGACCAACGTCG	57	BE230423: unknown protein
TATGTATGTACCTTAATTGTGT	52	D00642: chlorophyll a/b binding protein
Total Number of tags (different tags)	12,119 (7,546)	

*Tags represented as a 22-bp sequence excluding the NlaIII site (CATG).

[0112]

TABLE 4

A partial list of <i>Magnaporthe grisea</i> genes expressed in blast-infected rice leaves as revealed by SuperSAGE		
Tag sequence*	Count	Putative gene name**
CGATCACGAGGGGATGATGGTG	38	L20685: hydrophobin
TCAGACACAGGCTGTACAAGGC	2	nucleoside-diphosphate kinase
TCACGTTTAGAAAGGCGACCCG	2	60S ribosomal protein
TTGCCCGTATGTACATAAACAA	1	BM865406: NADH-ubiquinone oxidoreductase
CAATTGGTGTCTTCTTGGGTTT	1	AF056625: poly-ubiquitin
TCGTCTGTGGCTTCAGTTGCTG	1	unknown protein
ACGAGCTGATGCGCAAGGATGG	1	ABC transporter
Total number of putative <i>M. grisea</i> tags	74	

*Tags represented as a 22-bp sequence excluding the NlaIII site (CATG).

**Putative gene names were deduced from cDNA sequences or genome sequences of *M. grisea* matched with each tag.

[0113] To verify the results obtained by SuperSAGE, cDNA was synthesized by reverse-transcription from the same RNA as above using the following anchored Oligo-dT primer:

(SEQ ID NO: 8)
5'-GGCCACGCGTCGACTAGTACTTTTTTTTTTTTTTTT-3'.

[0114] With this cDNA as template, the 26-bp tags for hydrophobin, nucleosiddiphosphate lanase and 60S nrbsosomal protein genes (Table 4) were used for 3'-RACE together with the following primer:

5'-GGCCACGCGTCGACTAGTAC-3'. (SEQ ID NO: 9)

Specific PCR products were obtained for all the three genes (FIG. 10, left). The same primer sets were used for RT-PCR in mock-inoculated blast-susceptible cultivar (Kakehasbi), blast-inoculated susceptible cultivar (Kakehashi), mock-inoculated blast-resistant cultivar (Menomochi) and blast-inoculated resistant cultivar (Himenomochi). As expected, PCR products corresponding to the three blast genes were amplified only from susceptible cultivar inoculated with blast fungus (FIG. 10, right), demonstrating the authenticity of SuperSAGE annotation

[0115] Quantitative analysis of gene expression of two species in a sample as exemplified here could not be performed by the previous techniques. The use of 26-bp tags for gene expression analysis ("SuperSAGE") will enormously facilitate the gene expression analysis of two or more interacting organisms such as hosts and pathogens simultaneously.

Example 3

[0116] To demonstrate that SuperSAGE with 26-bp tags can be used for gene expression analysis of organisms for which DNA sequence data are not available, the following experiment was conducted. SuperSAGE was applied to gene expression analysis of a plant species, *Nicotiana benthamiana*, which was treated with either an protein elicitor INF1 from *Phytophthora infestans* (Kamoun et al. Mol. Plant-Microbe Interact. 10:13-20, 1997) or water. The leaves were collected one hour after infiltration of elicitor or water (flooding), and RNA was isolated therefrom.

[0117] More than 5000 tags were successfully isolated from each of the samples (Table 5). The ten most abundantly observed tags 15 in the Table 5 were used directly for 3'-RACE, and the resulting cDNA were sequenced. BLAST searches of the cDNAs identified the genes corresponding to the tags as given in Table 5.

TABLE 5

The 10 most frequently observed tags in <i>Nicotiana benthamiana</i> leaves revealed by SuperSAGE			
Tag Sequence*	flood-ing	INF1	Corresponding genes**
TGGAAGCTACCTATGTTCGGAT	130	73	ribulose bis-phosphate carboxylase
TGGAAGTTGCCTATGTTCGGAT	51	42	ribulose bis-phosphate carboxylase
TTCGGGTGCACTGATGCCACTC	32	33	ribulose bis-phosphate carboxylase

TABLE 5-continued

The 10 most frequently observed tags in <i>Nicotiana benthamiana</i> leaves revealed by SuperSAGE			
Tag Sequence*	flood- ing	IN1	Corresponding genes**
CAGCAAAGACCAAGAACAGCCC	16	22	oxygen-evolv- ing protein
TTCTCTATGTTCGGATTCTTTG	22	12	chlorophyll a/ b binding pro- tein
GTTATGGTGGCAGCCACCCCTAG	19	14	elicitor re- sponsive pro- tein
GCATCTTTGGCAACCCTTGCTG	13	11	photosystemII protein (Psa H)
TTTTCTATGTTCGGATTCTTTG	17	8	chlorophyll a/ b binding pro- tein
TTTAATCTCTAGTTACATACTG	15	7	photosystem I subunit PSI-E
TATCTTTTCAAGAATACTTTGT	13	5	photosystemII 10 kDa protein
Total number of tags	5,095	5,089	

*Tags represented as 22-bp sequences excluding the NlaIII site (CATG).

**Corresponding genes were deduced from the sequences of amplified cDNA fragments by 3'RACE using 26-bp tag sequence primer.

[0118] By comparing the frequency of each tag in INF1- and flooding-treated samples, tags were identified that were statistically significantly differentially represented in the two samples (Table 6). These tags were directly used for the 3'-RACE, and cDNA recovered was used for BLAST searches. This allowed annotation of most of the tags.

TABLE 6

Differentially expressed genes in INF1- and flooding-treated leaves of <i>Nicotiana benthamiana</i>			
Number of tags			
Tag sequence	flood- ing	INF1	genes
TTTTCTATGTTCGGATTCTTTG	17	8	chlorophyll a/b binding protein
AGGAATAGAGGGCAAGGTGCTC	11	6	phosphogly- cerate kinase (chloroplast)
GGCTTTTGCCACTAACTTTGTA	14	4	chlorophyll a/b binding protein
GAGCAATATGAAGACCACAGAG	11	3	alanine aminotrans- ferase

TABLE 6-continued

Differentially expressed genes in INF1- and flooding-treated leaves of <i>Nicotiana benthamiana</i>			
Number of tags			
Tag sequence	flood- ing	INF1	genes
GCTCTTGAAGAGGTTGTGAAAG	11	3	glycolate ox- idase
GGCAACAATGCTCTAGAGAAAG	10	2	ATP synthase (chloroplast)
CCTAGCTATTGACTACTGAAGT	10	2	unknown
GTTAAGGTTATTGCTTGGTATG	7	2	Glyceralde- hyde 3-phos- phate dehy- drogenase (chloro- plast))
TTTCCTTGACGATCACTCTTGG	7	2	PhotosystemII 23 kDa pro- tein
GTGATTCGCCGACGTAGCCGAAG	6	1	PhotosystemII protein
TTGCAACTTCTAGTCAATGACT	16	4	
ATGGCCAAGTAATTTACCATC	6	1	
AACTCATTAGAGACTCGAAGGG	6	0	
CAACACGAGCACGCACCTCTCT	0	7	unknown
TGCGGGATTTCGGTGGTGCCGGA	5	7	Ubiquitin conjugated protein
TTCGGGTGCACTGATGCCACTC	32	33	Ribulose bis- phosphate carboxylase

*Tags represented as 22 bp sequences excluding the NlaIII site (CATG).

**Corresponding genes were deduced from the sequences of amplified cDNA fragments by 3'RACE using 26-bp tag sequence primer.

[0119] The-26-bp tag sequences were used for RT-PCR together with the anchor primer to verify the results of SuperSAGE shown in Table 6. RT-PCR results (FIG. 11) clearly demonstrate that SuperSAGE results faithfully reflect the gene expression differences between the INF1 and flooding-treated samples.

[0120] The same 26-bp tag primer can be readily used for RT-PCR for kinetic study of each gene expression (FIG. 12). It was revealed that expression of four tested genes (genes for chlorophyll a/b binding protein, photosystem II protein, phosphoglycerate kinase, and ATP synthase) were shut off 15 min after the treatment with INF1.

[0121] This example demonstrates the great potential of SuperSAGE for gene expression analysis in the organisms for which no DNA sequence data is available. This potential comes from the sufficient size of tags for specific PCR primer and the fact that the gene expression profile obtained by SuperSAGE faithfully represents the real gene expression.

Free Text of Sequence Listing

[0122] SEQ ID NO: 1: RT-primer

[0123] SEQ ID NO: 2: First strand of Linker-A

[0124] SEQ ID NO: 3: Second strand of Linker-A

[0125] SEQ ID NO: 4: First strand of Linker-B

[0126] SEQ ID NO: 5: Second strand of Linker-B

[0127] SEQ ID NO: 6: Ditag primer (forward)

[0128] SEQ ID NO: 7: Ditag primer (reverse)

[0129] SEQ ID NO: 8: RT-primer

[0130] SEQ ID NO: 9: Primer for 3'-RACE

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 251

<210> SEQ ID NO 1

<211> LENGTH: 47

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Inventor: KAHL, Guenter; WINTER, Peter;
KRUEGER, Detlev; REICH, Stefanie; MATSUMURA, Hideo; TERAUCHI,
Ryohei

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:RT-primer

<400> SEQUENCE: 1

ctgatctaga ggtaccggat cccagcagtt tttttttttt ttttttt 47

<210> SEQ ID NO 2

<211> LENGTH: 46

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:first strand
of Linker-A

<400> SEQUENCE: 2

tttggatttg ctgggtgcagt acaactaggc ttaatacagc agcatg 46

<210> SEQ ID NO 3

<211> LENGTH: 42

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:second
strand of Linker-A

<400> SEQUENCE: 3

ctgctgtatt aagcctagtt gtactgcacc agcaaatcca aa 42

<210> SEQ ID NO 4

<211> LENGTH: 44

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:first strand
of Linker-B

<400> SEQUENCE: 4

tttctgctcg aattcaagct tctaacgatg tacgcagcag catg 44

<210> SEQ ID NO 5

<211> LENGTH: 40

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:second

-continued

strand of Linker-B

<400> SEQUENCE: 5

ctgctgcgta catcgtaga agcttgaatt cgagcagaaa 40

<210> SEQ ID NO 6

<211> LENGTH: 23

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Ditag primer
(forward)

<400> SEQUENCE: 6

caactaggct taatacagca gca 23

<210> SEQ ID NO 7

<211> LENGTH: 21

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Ditag primer
(reverse)

<400> SEQUENCE: 7

ctaacgatgt acgcagcagc a 21

<210> SEQ ID NO 8

<211> LENGTH: 37

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:RT-primer

<400> SEQUENCE: 8

ggccacgcgt cgactagtagc tttttttttt ttttttt 37

<210> SEQ ID NO 9

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:primer for
3'-RACE

<400> SEQUENCE: 9

ggccacgcgt cgactagtagc 20

<210> SEQ ID NO 10

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 10

gatccgtctc tctgggagga at 22

<210> SEQ ID NO 11

<211> LENGTH: 10

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

-continued

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 11

gatccgtctc 10

<210> SEQ ID NO 12
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 12

ttgtaatact ccatcaaaga gt 22

<210> SEQ ID NO 13
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 13

ttgtaatact 10

<210> SEQ ID NO 14
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 14

cgaaatcgat tccgagttct ct 22

<210> SEQ ID NO 15
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 15

cgaaatcgat 10

<210> SEQ ID NO 16
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 16

atggcctgag gaagtggctc gc 22

<210> SEQ ID NO 17
<211> LENGTH: 10
<212> TYPE: DNA

-continued

<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 17

atggcctgag 10

<210> SEQ ID NO 18
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 18

atgatgatat actacacttg at 22

<210> SEQ ID NO 19
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 19

atgatgatat 10

<210> SEQ ID NO 20
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 20

ttcggcttct tcgtccaggc ca 22

<210> SEQ ID NO 21
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 21

ttcggcttct 10

<210> SEQ ID NO 22
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 22

tttghtaactc gttatatcct ca 22

<210> SEQ ID NO 23

-continued

<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 23

tttgtaactc 10

<210> SEQ ID NO 24
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 24

ctcaagatga tcgaggacta cc 22

<210> SEQ ID NO 25
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 25

ctcaagatga 10

<210> SEQ ID NO 26
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 26

tatatttatac cataatatac tc 22

<210> SEQ ID NO 27
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 27

tatatttatac 10

<210> SEQ ID NO 28
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 28

taatggtaca tatctccttg tt 22

-continued

<210> SEQ ID NO 29
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 29

taatgttaca 10

<210> SEQ ID NO 30
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 30

acgattggtg aatacccttg ga 22

<210> SEQ ID NO 31
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 31

acgattggtg 10

<210> SEQ ID NO 32
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 32

ttgtgattca taactctagt ag 22

<210> SEQ ID NO 33
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 33

ttgtgattca 10

<210> SEQ ID NO 34
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 34

-continued

tacataaatg gaaaaagaga gg 22

<210> SEQ ID NO 35
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 35

tacataaatg 10

<210> SEQ ID NO 36
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 36

tttgacaaga cattttcagt at 22

<210> SEQ ID NO 37
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 37

tttgacaaga 10

<210> SEQ ID NO 38
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 38

gaatcaatac tgtaactatt at 22

<210> SEQ ID NO 39
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 39

gaatcaatac 10

<210> SEQ ID NO 40
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

-continued

<400> SEQUENCE: 40

ttcatatgaa ataaaattat ga

22

<210> SEQ ID NO 41

<211> LENGTH: 10

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 41

ttcatatgaa

10

<210> SEQ ID NO 42

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 42

gggggaggca agccgacgat cg

22

<210> SEQ ID NO 43

<211> LENGTH: 10

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 43

gggggaggca

10

<210> SEQ ID NO 44

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 44

tatgtatgta ccttaattgt gt

22

<210> SEQ ID NO 45

<211> LENGTH: 10

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 45

tatgtatgta

10

<210> SEQ ID NO 46

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic

-continued

DNA (Tag Sequence)

<400> SEQUENCE: 46

tggtactagc tgctactgtc ta

22

<210> SEQ ID NO 47

<211> LENGTH: 10

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 47

tggtactagc

10

<210> SEQ ID NO 48

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 48

gctctttctg tttgagggcc at

22

<210> SEQ ID NO 49

<211> LENGTH: 10

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 49

gctctttctg

10

<210> SEQ ID NO 50

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 50

gctgtgttgt atctaaactg tt

22

<210> SEQ ID NO 51

<211> LENGTH: 10

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 51

gctgtgttgt

10

<210> SEQ ID NO 52

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

-continued

<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 52
taaacaaacc ttctaagaga ca 22

<210> SEQ ID NO 53
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 53
taaacaaacc 10

<210> SEQ ID NO 54
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 54
tcgtggggta tctagggctg ga 22

<210> SEQ ID NO 55
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 55
tcgtggggta 10

<210> SEQ ID NO 56
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 56
agcattgttt tacacgtaat gc 22

<210> SEQ ID NO 57
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 57
agcattgttt 10

<210> SEQ ID NO 58
<211> LENGTH: 22

-continued

<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 58

aagctacttg cttcccttg tc 22

<210> SEQ ID NO 59
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 59

aagctacttg 10

<210> SEQ ID NO 60
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 60

ggtttcagct tggtcgacta at 22

<210> SEQ ID NO 61
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 61

ggtttcagct 10

<210> SEQ ID NO 62
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 62

atgtagatcg atggatgtac ca 22

<210> SEQ ID NO 63
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 63

atgtagatcg 10

-continued

<210> SEQ ID NO 64
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 64

catatgttga tcaatctgga gg 22

<210> SEQ ID NO 65
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 65

catatgttga 10

<210> SEQ ID NO 66
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 66

taacttactc ttggttaatt at 22

<210> SEQ ID NO 67
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 67

taacttactc 10

<210> SEQ ID NO 68
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 68

tgtaactcac tggattggag tg 22

<210> SEQ ID NO 69
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 69

tgtaactcac 10

-continued

<210> SEQ ID NO 70
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 70
gtaattgtcc tgggtgattt ct 22

<210> SEQ ID NO 71
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 71
gtaattgtcc 10

<210> SEQ ID NO 72
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 72
ctggtattct gcaacgttac at 22

<210> SEQ ID NO 73
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 73
ctggtattct 10

<210> SEQ ID NO 74
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 74
ggaaaagcta ctgactggta at 22

<210> SEQ ID NO 75
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 75

-continued

ggaaaagcta 10

<210> SEQ ID NO 76
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 76

ggacaatgtc acttggttca ca 22

<210> SEQ ID NO 77
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 77

ggacaatgtc 10

<210> SEQ ID NO 78
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 78

ataattattg ctggatgac aa 22

<210> SEQ ID NO 79
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 79

ataattattg 10

<210> SEQ ID NO 80
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 80

ttcatcagca tattttctaa ta 22

<210> SEQ ID NO 81
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

-continued

<400> SEQUENCE: 81

ttcatcagca

10

<210> SEQ ID NO 82

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 82

gatcattgct tgatggtaaa ga

22

<210> SEQ ID NO 83

<211> LENGTH: 10

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 83

gatcattgct

10

<210> SEQ ID NO 84

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 84

cgggtgctcc tacatctctt ct

22

<210> SEQ ID NO 85

<211> LENGTH: 10

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 85

cgggtgctcc

10

<210> SEQ ID NO 86

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 86

gtccgcgcc cctgcgtcgt cg

22

<210> SEQ ID NO 87

<211> LENGTH: 10

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

-continued

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 87

gtccgcgccc 10

<210> SEQ ID NO 88
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 88

tatcaatgta tttttatctg ta 22

<210> SEQ ID NO 89
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 89

tatcaatgta 10

<210> SEQ ID NO 90
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 90

taataatctg taacctgaag ac 22

<210> SEQ ID NO 91
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 91

taataatctg 10

<210> SEQ ID NO 92
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 92

tagagttaga gatattgag ct 22

<210> SEQ ID NO 93
<211> LENGTH: 10
<212> TYPE: DNA

-continued

<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 93
tagagttaga 10

<210> SEQ ID NO 94
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 94
cgattttgag taaaatacgc ta 22

<210> SEQ ID NO 95
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 95
cgattttgag 10

<210> SEQ ID NO 96
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 96
ggccgcctcc cgtgtgttcg tg 22

<210> SEQ ID NO 97
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 97
ggccgcctcc 10

<210> SEQ ID NO 98
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 98
atatatcaca atgtctgatc ca 22

<210> SEQ ID NO 99

-continued

<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 99

atatatcaca 10

<210> SEQ ID NO 100
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 100

gtcccagctt actgcaagat ca 22

<210> SEQ ID NO 101
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 101

gtcccagctt 10

<210> SEQ ID NO 102
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 102

gtcgcctctc tgctcgtgcc cc 22

<210> SEQ ID NO 103
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 103

gtcgcctctc 10

<210> SEQ ID NO 104
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 104

cgagaatgct aatgggcgat ca 22

-continued

<210> SEQ ID NO 105
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 105

cgagaatgct 10

<210> SEQ ID NO 106
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 106

gcggcggcga tggtcctccc cg 22

<210> SEQ ID NO 107
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 107

gcggcggcga 10

<210> SEQ ID NO 108
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 108

agatactgta caggattgaa tg 22

<210> SEQ ID NO 109
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 109

agatactgta 10

<210> SEQ ID NO 110
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 110

-continued

gccgccacct cgctgtcgcc gc 22

<210> SEQ ID NO 111
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 111

gccgccacct 10

<210> SEQ ID NO 112
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 112

cctaacttgc tgtaagcaca tt 22

<210> SEQ ID NO 113
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 113

cctaacttgc 10

<210> SEQ ID NO 114
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 114

gcaagttacc aaatggaaaa ta 22

<210> SEQ ID NO 115
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 115

gcaagttacc 10

<210> SEQ ID NO 116
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

-continued

<400> SEQUENCE: 116

atatatatat aaaaaggatt ag

22

<210> SEQ ID NO 117

<211> LENGTH: 10

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 117

atatatatat

10

<210> SEQ ID NO 118

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 118

caggccgtcg cgctcgacgt gc

22

<210> SEQ ID NO 119

<211> LENGTH: 10

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 119

caggccgtcg

10

<210> SEQ ID NO 120

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 120

aaccaggatt caccagttct gg

22

<210> SEQ ID NO 121

<211> LENGTH: 10

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 121

aaccaggatt

10

<210> SEQ ID NO 122

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic

-continued

DNA (Tag Sequence)

<400> SEQUENCE: 122

tatatgtttt ctagttttct ct 22

<210> SEQ ID NO 123

<211> LENGTH: 10

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 123

tatatgtttt 10

<210> SEQ ID NO 124

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 124

taacaagcag attagaaagc tg 22

<210> SEQ ID NO 125

<211> LENGTH: 10

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 125

taacaagcag 10

<210> SEQ ID NO 126

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 126

cagcaatact ctgtaatat at 22

<210> SEQ ID NO 127

<211> LENGTH: 10

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 127

cagcaatact 10

<210> SEQ ID NO 128

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

-continued

<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 128
agtgactgtt gttgtagaga ac 22

<210> SEQ ID NO 129
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 129
agtgactgtt 10

<210> SEQ ID NO 130
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 130
gcttgagccc tggggccctg gc 22

<210> SEQ ID NO 131
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 131
gcttgagccc 10

<210> SEQ ID NO 132
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 132
aagtgttata ctgtacactg at 22

<210> SEQ ID NO 133
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 133
aagtgttata 10

<210> SEQ ID NO 134
<211> LENGTH: 22

-continued

<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 134

tgtaactaaa tgaaatggtg tg 22

<210> SEQ ID NO 135
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 135

tgtaactaaa 10

<210> SEQ ID NO 136
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 136

gagaaaatta acgtgaaaaa aa 22

<210> SEQ ID NO 137
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 137

gagaaaatta 10

<210> SEQ ID NO 138
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 138

tttgatgctt gacaggcaac at 22

<210> SEQ ID NO 139
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 139

tttgatgctt 10

-continued

<210> SEQ ID NO 140
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 140

gctattgcaa ctgtggtgac tg 22

<210> SEQ ID NO 141
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 141

gctattgcaa 10

<210> SEQ ID NO 142
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 142

taacaagcga ggtgcttgtg tc 22

<210> SEQ ID NO 143
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 143

taacaagcga 10

<210> SEQ ID NO 144
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 144

ccaatactgg tgaaaaaaaa aa 22

<210> SEQ ID NO 145
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 145

ccaatactgg 10

-continued

<210> SEQ ID NO 146
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 146
gaactttggg atgtaagcac tg 22

<210> SEQ ID NO 147
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 147
gaactttggg 10

<210> SEQ ID NO 148
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 148
tagcaatgcc agggatttgt at 22

<210> SEQ ID NO 149
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 149
tagcaatgcc 10

<210> SEQ ID NO 150
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 150
ggatattgga atgggagtat aa 22

<210> SEQ ID NO 151
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 151

-continued

ggatattgga 10

<210> SEQ ID NO 152
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 152

ctcgacttcg ccggcgagca cg 22

<210> SEQ ID NO 153
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 153

ctcgacttcg 10

<210> SEQ ID NO 154
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 154

gcacccacga gagagggggc tc 22

<210> SEQ ID NO 155
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 155

gcacccacga 10

<210> SEQ ID NO 156
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 156

gacgaggacg ccccggcggg tg 22

<210> SEQ ID NO 157
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

-continued

<400> SEQUENCE: 157

gacgaggacg

10

<210> SEQ ID NO 158

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 158

tgttagtatt atgtaactct gt

22

<210> SEQ ID NO 159

<211> LENGTH: 10

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 159

tgttagtatt

10

<210> SEQ ID NO 160

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 160

gccgccgagc cggagatacc gg

22

<210> SEQ ID NO 161

<211> LENGTH: 10

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 161

gccgccgagc

10

<210> SEQ ID NO 162

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 162

ggatatgcttc cattgtcagc ag

22

<210> SEQ ID NO 163

<211> LENGTH: 10

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

-continued

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 163

ggtatgcttc 10

<210> SEQ ID NO 164
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 164

taattttaat catctgtcag gc 22

<210> SEQ ID NO 165
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 165

taattttaat 10

<210> SEQ ID NO 166
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 166

gatatggacg agtgtgttat tt 22

<210> SEQ ID NO 167
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 167

gatatggacg 10

<210> SEQ ID NO 168
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 168

tgtaaactt gccattctga ta 22

<210> SEQ ID NO 169
<211> LENGTH: 10
<212> TYPE: DNA

-continued

<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 169

tgttaaactt 10

<210> SEQ ID NO 170
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 170

ttgtcaaggg ccagttggat gt 22

<210> SEQ ID NO 171
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 171

ttgtcaaggg 10

<210> SEQ ID NO 172
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 172

gcacctgaca ccgacactga ag 22

<210> SEQ ID NO 173
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 173

gcacctgaca 10

<210> SEQ ID NO 174
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 174

ggatgccact ggatcgaaaa ga 22

<210> SEQ ID NO 175

-continued

<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 175

ggatgccact 10

<210> SEQ ID NO 176
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 176

gatagcaagt agtagcctgt tt 22

<210> SEQ ID NO 177
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 177

gatagcaagt 10

<210> SEQ ID NO 178
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 178

caatccttga tccgaactgt gc 22

<210> SEQ ID NO 179
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 179

caatccttga 10

<210> SEQ ID NO 180
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 180

acccgagtgt cggctcttcag tt 22

-continued

<210> SEQ ID NO 181
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 181

acccgagtgt 10

<210> SEQ ID NO 182
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 182

ggagcttgac atcaatggca ga 22

<210> SEQ ID NO 183
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 183

ggagcttgac 10

<210> SEQ ID NO 184
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 184

cagcggcggc ttaggcgcgt gg 22

<210> SEQ ID NO 185
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 185

cagcggcggc 10

<210> SEQ ID NO 186
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 186

-continued

gaacccaaaat tttcagagac cc 22

<210> SEQ ID NO 187
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 187

gaacccaaaat 10

<210> SEQ ID NO 188
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 188

cctcacggca tcttaactag aa 22

<210> SEQ ID NO 189
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 189

cctcacggca 10

<210> SEQ ID NO 190
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 190

accatattct tgttgtactg cc 22

<210> SEQ ID NO 191
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 191

accatattct 10

<210> SEQ ID NO 192
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

-continued

<400> SEQUENCE: 192

ttcttgataa gggatcgatt ag

22

<210> SEQ ID NO 193

<211> LENGTH: 10

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 193

ttcttgataa

10

<210> SEQ ID NO 194

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 194

tactactacc ttgtaaactt tt

22

<210> SEQ ID NO 195

<211> LENGTH: 10

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 195

tactactacc

10

<210> SEQ ID NO 196

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 196

gttgagcagg agctgccttc tg

22

<210> SEQ ID NO 197

<211> LENGTH: 10

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 197

gttgagcagg

10

<210> SEQ ID NO 198

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic

-continued

DNA (Tag Sequence)

<400> SEQUENCE: 198

ttcggctgca ccgatgccac cc 22

<210> SEQ ID NO 199
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 199

ttcggctgca 10

<210> SEQ ID NO 200
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 200

gccggtgggc aggagcaagc gg 22

<210> SEQ ID NO 201
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 201

gccggtgggc 10

<210> SEQ ID NO 202
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 202

tagtaggaag ttgacatctt ac 22

<210> SEQ ID NO 203
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 203

tagtaggaag 10

<210> SEQ ID NO 204
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

-continued

<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 204
agggccggcg gcaagcggcg gg 22

<210> SEQ ID NO 205
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 205
agggccggcg 10

<210> SEQ ID NO 206
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 206
caggcagaca gatccgtgtg ta 22

<210> SEQ ID NO 207
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 207
caggcagaca 10

<210> SEQ ID NO 208
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 208
ttcggcttct tcgtccaggc ca 22

<210> SEQ ID NO 209
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 209
gatccgtctc tctgggagga at 22

<210> SEQ ID NO 210
<211> LENGTH: 22

-continued

<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 210

gcgacgcac gccttcagct aa 22

<210> SEQ ID NO 211
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 211

tggtggctta gctctacgtg ta 22

<210> SEQ ID NO 212
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 212

tcggacaagt gcggcaactg cg 22

<210> SEQ ID NO 213
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 213

ttgtaatact ccatcaaaga gt 22

<210> SEQ ID NO 214
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 214

aattgagttc gctttgggta tg 22

<210> SEQ ID NO 215
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 215

atgatgatat actacacttg at 22

-continued

<210> SEQ ID NO 216
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 216
gcgtccacgc tgaccaacgt cg 22

<210> SEQ ID NO 217
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 217
tatgtatgta ccttaattgt gt 22

<210> SEQ ID NO 218
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 218
cgatcacgag gggatgatgg tg 22

<210> SEQ ID NO 219
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 219
tcagacacag gctgtacaag gc 22

<210> SEQ ID NO 220
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 220
tcacgtttag aaaggcgacc cg 22

<210> SEQ ID NO 221
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 221
ttgccgtat gtacataaac aa 22

-continued

<210> SEQ ID NO 222
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 222

caattggtgt ttctttgggt tt 22

<210> SEQ ID NO 223
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 223

tcgtctgtgg cttcagttgc tg 22

<210> SEQ ID NO 224
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 224

acgagctgat gcgcaaggat gg 22

<210> SEQ ID NO 225
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 225

tggaagctac ctatgttcgg at 22

<210> SEQ ID NO 226
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 226

tggaagttgc ctatgttcgg at 22

<210> SEQ ID NO 227
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 227

-continued

ttcgggtgca ctgatgccac tc 22

<210> SEQ ID NO 228
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 228

cagcaaagac caagaacagc cc 22

<210> SEQ ID NO 229
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 229

ttctctatgt tcggattctt tg 22

<210> SEQ ID NO 230
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 230

gttatgttg cagccaccct ag 22

<210> SEQ ID NO 231
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 231

gcatcttttg caacccttgc tg 22

<210> SEQ ID NO 232
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 232

ttttctatgt tcggattctt tg 22

<210> SEQ ID NO 233
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

-continued

<400> SEQUENCE: 233

tttaatctct agttacatac tg

22

<210> SEQ ID NO 234

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 234

tatcttttca agaatacttt gt

22

<210> SEQ ID NO 235

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 235

ttttctatgt tcggattctt tg

22

<210> SEQ ID NO 236

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 236

aggaatagag ggcaagggtgc tc

22

<210> SEQ ID NO 237

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 237

ggcttttgcc actaactttg ta

22

<210> SEQ ID NO 238

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 238

gagcaatatg aagaccacag ag

22

<210> SEQ ID NO 239

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

-continued

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 239

gctcttgaag aggttgtaa ag 22

<210> SEQ ID NO 240

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 240

ggcaacaatg ctctagagaa ag 22

<210> SEQ ID NO 241

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 241

cctagctatt gactactgaa gt 22

<210> SEQ ID NO 242

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 242

gttaaggta ttgcttgga tg 22

<210> SEQ ID NO 243

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 243

tttccttgac gatcactctt gg 22

<210> SEQ ID NO 244

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 244

gtgattcccg acgtagccga ag 22

<210> SEQ ID NO 245

<211> LENGTH: 22

<212> TYPE: DNA

-continued

<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 245
ttgcaacttc tagtcaatga ct 22

<210> SEQ ID NO 246
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 246
atggccaagt aatttcacca tc 22

<210> SEQ ID NO 247
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 247
aactcattag agactcgaag gg 22

<210> SEQ ID NO 248
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 248
caacacgagc acgcacctct ct 22

<210> SEQ ID NO 249
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 249
tgcgggattc ggtggtgccg ga 22

<210> SEQ ID NO 250
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:Synthetic
DNA (Tag Sequence)

<400> SEQUENCE: 250
ttcgggtgca ctgatgccac tc 22

<210> SEQ ID NO 251

-continued

```

<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Linker sequence

```

```

<400> SEQUENCE: 251

```

```

cagcagcatg

```

10

1. Use of a type III restriction enzyme to isolate from cDNA of an expressed gene a tag comprising more than 25 nucleotides and capable of identifying the expressed gene, wherein the 3' end of the tag is defined by a cleavage site of the type III restriction enzyme and the 5' end of the tag is defined by the cleavage site of another restriction enzyme that is closest to the 3' end of the cDNA of the expressed gene.

2. The use of claim 1, wherein the type III restriction enzyme is EcoP15I.

3. The use of claim 2, wherein the other restriction enzyme is NlaIII.

4. A tag comprising more than 25 nucleotides and capable of identifying a expressed gene, wherein the 3' end of the tag is defined by a cleavage site of a type III restriction enzyme and the 5' end of the tag is defined by the cleavage site of another restriction enzyme that is closest to the 3' end of the cDNA of the expressed gene.

5. The tag of claim 4, wherein the type III restriction enzyme is EcoP15I.

6. The tag of claim 5, wherein the other restriction enzyme is NlaIII.

7. A Ditag-oligonucleotide comprising two tags each of which is derived from a different expressed gene, wherein

each tag comprises more than 25 nucleotides and is capable of identifying an expressed gene, and the 3' end of the tag is defined by a cleavage site of a type III restriction enzyme and the 5' end of the tag is defined by a cleavage site of another restriction enzyme that is closest to the 3' end of the cDNA of the expressed gene.

8. The Ditag-oligonucleotide of claim 7 produced by a method comprising the following steps:

- 1) synthesizing a cDNA pool from mRNA of expressed genes using a primer comprising oligo-dT and a recognition sequence of the type III restriction enzyme, followed by digestion of the cDNA pool with another restriction enzyme;
- 2) purifying fragments comprising poly A sequence from the above cDNA pool, and ligating the fragments to either linker-A or linker-B, both of which comprise the recognition sequence of the type III restriction enzyme;
- 3) digesting the above fragments with the type III restriction enzyme, and ligating the resulting fragment comprising linker-A to the resulting fragment comprising linker-B after performing a 3'-filling reaction; and
- 4) digesting the above ligated fragments with the other restriction enzyme of step 1) to cleave off the linker sequence, and thereby obtain the ditag-oligonucleotide.

9. The ditag-oligonucleotide of claim 7, wherein the ditag-oligonucleotide is made by a random association of two tags derived from the different expressed genes.

10. The ditag-oligonucleotide of claim 7, wherein the type III restriction enzyme is EcoP15I.

11. The ditag-oligonucleotide of claim 10, wherein the other restriction enzyme is NlaIII.

12. The ditag-oligonucleotide of claim 11, wherein the Linker-A and Linker-B are double-stranded DNA which differ from each other and are made by annealing the following first strand of DNA(1) and second strand of DNA(2);

DNA (1) : 5' -N₃₀₋₄₀-CAGCAGCATG-3'

DNA (2) : 3' -N₃₀₋₄₀-GTCGTC-5'

wherein, N₃₀₋₄₀ of DNA(1) and N₃₀₋₄₀ of DNA(2) are arbitrary nucleotide sequences from 30 to 40 which are complementary to each other, and wherein the 5' end of DNA(1) may be labeled and the 3' end of DNA(2) may be amino-modified.

13. A polynucleotide comprising at least two ditag-oligonucleotides of claim 7.

14. The polynucleotide of claim 13, wherein the polynucleotide comprises 2 to 200 ditag-oligonucleotides.

15. A method of gene expression analysis comprising:

analyzing the nucleotide sequence of the polynucleotide of claim 13, and

quantifying the expression level of an expressed gene based on the number of tags corresponding to the expressed gene included in the polynucleotide.

16. A method of gene expression analysis comprising the following steps of;

- 1) synthesizing a cDNA pool from mRNA of expressed genes using a primer comprising oligo-dT and a recognition sequence of a type III restriction enzyme, followed by digestion of the cDNA pool with another restriction enzyme;
- 2) purifying fragments comprising poly A sequence from the above cDNA pool, and ligating the fragments to either linker-A or linker-B both of which comprise the recognition sequence of the type III restriction enzyme;
- 3) digesting the above fragments with the type III restriction enzyme, and ligating the resulting fragment comprising linker-A to the resulting fragment comprising linker-B after performing a 3'-filling reaction; and

4) digesting the above ligated fragments with the other restriction enzyme of step 1) to cleave off the linker sequence, and thereby obtain a ditag-oligonucleotide comprising two tags of more than 25 nucleotides and capable of identifying the expressed gene;

5) ligating the ditag-oligonucleotides to produce a polynucleotide;

6) analyzing the nucleotide sequence of the above polynucleotide, and quantifying the expression level of a expressed gene based on the number of tags corresponding to the expressed gene included in the polynucleotide.

17. The method of claim 16, wherein the type III restriction enzyme is EcoP15I.

18. The method of claim 17, wherein the other restriction enzyme is NlaIII.

19. The method of claim 18, wherein the Linker-A and Linker-B are double-stranded DNA which differ from each other and are made by annealing the following first strand of DNA(1) and second strand of DNA(2);

DNA (1) : 5' -N₃₀₋₄₀-CAGCAGCATG-3'

DNA (2) : 3' -N₃₀₋₄₀-GTCGTC-5'

wherein, N₃₀₋₄₀ of DNA(1) and N₃₀₋₄₀ DNA(2) are arbitrary nucleotide sequences from 30 to 40 which are complementary to each other, and wherein the 5' end of DNA(1) may be labeled and the 3' end of DNA(2) may be amino-modified.

20. A kit for isolating a tag comprising more than 25 nucleotides and capable of identifying an expressed gene, comprising the following elements:

a) An RT primer defined by the sequence 5'-N₁₈₋₂₅-CAG-CAG-T₁₅₋₂₅-3',

wherein N₁₈₋₂₅ is arbitrary nucleotide sequence from 18 to 25 not comprising a sequence 5'-CAGCAG-3' and a sequence 5'-CATG-3', and wherein the 5' end of RT-primer may be biotinylated;

b) Linker-A and Linker-B which are double-stranded DNA different from each other and made by annealing the following first strand of DNA(1) and second strand of DNA(2):

DNA (1) : 5' -N₃₀₋₄₀-CAGCAGCATG-3'

DNA (2) : 3' -N₃₀₋₄₀-GTCGTC-5'

wherein, N₃₀₋₄₀ of (1) and N₃₀₋₄₀ of (2) are arbitrary nucleotide sequences from 30 to 40 which are complementary to each other, and

the 5' end of DNA(1) may be labeled and the 3' end of DNA(2) may be amino-modified;

c) primers capable of hybridizing to the above Linker-A or Linker-B.

21. The kit of claim 20, further comprising EcoP15I and/or NlaIII.

* * * * *